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HAMILTON B. G. ROBINSON

AUGUST, 1950

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THE EDITOR'S VIEWPOINT

Engel, Ray, and Orban (p. 410) used histochemical methods to investigate the tissue changes in desquamative gingivitis. They found disturbances in the ground substance of the gingival connective tissue, with absence or partial loss of the basement membrane and increased water-soluble glycoprotein and water-soluble-alcohol-insoluble glycoprotein residues. These, they propose, are partly the result of increased production of depolymerizing enzymes. Their findings are compatible with the clinical observation of separation of the epithelium by air blown into the gingival sulcus and, probably, with the earlier suggestion of a relationship of female sex hormones to the disease, although the disturbance is not limited to females.

Linghorne and O'Connell (p. 419) detached the gingivae of dogs by surgery, removed sections of bone and periodontal membrane, and sutured the flap in place with and without scaling. Under these experimental conditions, formation of new cementum and reattachment of connective tissue to the tooth surface was microscopically observed. Although these encouraging signs took place under certain advantageous conditions, i.e., the pocket had been formed surgically, not by trauma and infection, there were certain adverse factors, i.e., lack of treatment by packs. The question of reattachment of the connective tissue has too long been argued at the level of opinion. The approach from the viewpoint of pathologic and physiologic possibility and by the experimental method is to be commended.

From the studies of the oxygen consumption of healing gingival tissues, Glickman, Turesky, and Manhold (p. 429) could find no evidence to suggest that the introduction of oxygen during healing appreciably hastens the normal cellular processes. The increased demand for oxygen during healing is apparently met by increased blood supply and by increasing the quantity of oxygen taken from a given volume of blood. The utilization of the Warburg technic to investigate an old clinical problem in this instance is suggestive of the many applications of basic technics to the solution of clinical problems.

Bartels (p. 436) has demonstrated the formation of crystals, in vitro, about and in colonies of oral filamentous organisms. This work adds evidence to Naeslund's theory of calculus formation as a metabolic activity of oral actinomycetes. Since these same types of organisms appear to form the skeleton of dentobacterial plaques, which are the regions of the acid activity which initiates caries, they may well play a second role of calculus formers in the presence of alkaline products.

Baume (p. 440), in studying the biogenesis of overbite, came to the conclusion (from the studies of 52 cases) that the degree of incisor overbite in the permanent dentition is determined by the extent of mandibular oral growth which occurred during the eruption of successional teeth. These studies of Baume should be considered in devising preventive treatment for possible orthodontic disturbances.

Manly and Braley (p. 448) report on a study of masticatory efficiency. While this paper is essentially an account and evaluation of the method, it offers some data on relative efficiency of complete and mutilated natural dentitions and of artificial dentures. One point mentioned, the probable tendency to select less tough foods to compensate for the relative inefficiency of artificial dentures and the likelihood, thereby, of reducing amino acid and vitamin intake, is substantiated clinically. Frequently, lesions attributable to vitamin B complex deficiency and aggravated by denture trauma are seen in mouths of new denture wearers. The soreness tends to force selection of even softer and less nutritionally effective foods with resultant augmentation of the original cause. For this reason it probably is wise to supplement the food intake of new denture patients during the construction and postconstruction period with vitamin B complex.

Neumann (p. 463) presents another approach to the problem of masticatory function. His technic utilizes the current produced by muscular activity in the process of chewing to determine the forces applied to various foods. He found surprisingly little force used in the chewing of most foods

available in our economy. As Neumann states, it is time to stop making statements about the effects of masticatory forces on caries, as well as on periodontal disease and denture effectiveness, without objective measurements of the forces involved.

Landgren and Peyton (p. 469) report studies on hygroscopic expansion of casting investments, in which they found immersion of the specimen 2 to 12 minutes after mixing and keeping it in water at 37° C. for 50 minutes to be the desirable technic. These continued physical studies on materials used in restorative dentistry must be pursued along with biological studies of the tissues being restored and with efforts to prevent the need for restorative dentistry. The latter Utopian goal, while the ultimate aim of dental science, must await discovery of methods for prevention of caries and periodontal diseases and then development of means of supplying such methods to every person.

McDonald and Phillips (p. 482) had a special alloy formulated to produce shrinkage in amalgam fillings far beyond the limit given in the specifications accepted by the American Dental Association. Three years after teeth of children were filled with amalgam made with this alloy no clinical evidence of open margins or "recurrent caries" was observed. The authors point out that this is not to be construed as recommendation for use of unaccepted alloys or for deviation from careful technic; it only indicates that contraction from slight overtrituration or other causes will not be clinically observable. Such carry over and correlation of physical, as well as biological, studies with clinical observation is highly important.

Taylor and Frank (p. 486) investigated the properties of "self-curing" resins and drew rather mixed conclusions. They believe it possible to make such resins color stable but point out that the mechanical properties are below those of resins used in dentures. Since the degree of polymerization is lower than desired, and since it has been suggested that a contact stomatitis may be due to excess monomer rather than to sensitivity to the polymerized resin, the possibility of reactions of the mucosa contracting these low temperature polymerized resins should be considered.

It is well recognized that heat may have an adverse effect on the dental pulp and that cutting with various instruments, and the setting of cements, silicates, and resinous filling materials, create heat. For these reasons the tolerance of the pulp tissue to heat and the thermal conductivity of dentin are important. Lisanti and Zander (p. 493) developed a method for determining heat conductivity of dentin. They found its thermal conductivity similar to that of glass and closer to that of zinc phosphate cement than to those of metallic filling materials.

Zipkin and Piez (p. 498) were unable to find significant differences between the citrate content of enamel and dentin from sound and from carious teeth. The quantities of citrate found compared closely with those reported by Free. The possible relationship between citrate and erosion and dental caries has stimulated studies of citrate of saliva, dentin, and enamel. While this may prove to be a blind alley, none of the information gained will be lost but will all add to our sum total of important basic knowledge.

Using filtered and whole salivas with carbohydrate, Sreebny, Kirch, and Kesel (p. 506) found evidence indicating that the bacterial enzyme systems involved in carbohydrate degradation are intracellular. They believe that this indicates that methods designed to alter the ability of saliva to degrade glucose should be aimed at destruction of the metabolism of the microorganisms concerned.

Orland, Hemmens, and Harrison (p. 512) fed diets deficient in certain factors to hamsters, supplying the missing factors by parenteral injections. Thus the diet (food intake) of the animals was deficient as it passed through the oral cavity, although the hamster's nutrition was adequate. The study, in effect, was one of the effects of certain dietary factors of the oral bacteria on caries production in vivo. While only trends were noted, the general approach may offer valuable information. Animals deficient in vitamin K or choline chloride, nicotinic acid, or inositol showed increased caries. Those deficient in vitamin E reduced progress of caries, and those without nicotinic acid reduced caries. Among the points that should be investigated in fur-

ther studies are the oral flora in its day-to-day relationships and the vitamin content of the saliva.

Shourie, Hein, and Hodge (p. 529) found that 40 ppm of fluoride as sodium monofluorophosphate and as sodium fluoride in the drinking water of Syrian hamsters produced almost maximal reduction in caries, yet the sodium monofluorophosphate is considerably less toxic and does not yield fluoride ion in aqueous solution. These findings again indicate that we have much to learn of the modus operandi of fluorides in caries control and, at the same time, the most effective way of using this caries-fluoride relationship.

Hill, Blayney, and Wolf (p. 534) compare the prefluoride and postfluoride caries experience of 6-, 7-, and 8-year-olds in the Evanston area. They carefully point out that differences in caries experience after one year may represent only normal trends or may be influenced by such factors as increased tooth brushing, use of therapeutic dentifrices, or lowering of refined carbohydrate consumption. While some enthusiasts might cite the data reported as acceptable evidence of the effectiveness of fluoridation of water supplies, the authors conclude that it cannot be assumed that the changes are necessarily due to additions of fluorides.

Dunning (p. 541) discusses statistical analysis of caries data. His statement, "the first job in the elimination of errors lies in the field of common accuracy rather than mathematics," bears repetition. We could never understand how data could be compared giving equal weight to careful exacting examination by astute clinicians aided by the roentgenogram and the best lighting conditions and to "folding chair" examinations with tongue blades or flashlights in a "sunny" corner. No amount of statistical analysis will remove the inaccuracies introduced by inexact methods anymore than a good mathematician could expect to present a correct inventory based on counts made by a tribe of monkeys. Dunning's paper deserves study and thought by all those attempting to evaluate caries control methods.

Adler, Straub, and Popovics (p. 549) have devised an ingenious method for studying the uptake of fluorides by enamel in vivo. It is apparent that it offers many advantages over in vitro methods now in use. The authors found that there is a greater uptake of fluorides after initial prophylaxis, that longer exposure results in greater uptake of fluorides, and that individuals over 20 years of age show less uptake than those under 18 years of age. They could not demonstrate any advantage in increasing the concentration of fluorides.

Scott and Wyckoff (p. 556) show beautiful photomicrographs of dentinal structures as seen with the aid of the electron microscope. The odontoblastic fibers appear as thin walled tubes and intercommunication of their branches is evident. The pattern of the matrix fibrils resembles that of collagen. A third type of fiber is also shown. During the development of electron microscopy, all interpretations must be made with great care, but study of the region beyond the resolving power of ordinary microscopy offers great promise.

H. B. G. R.

THE PATHOGENESIS OF DESQUAMATIVE GINGIVITIS: A DISTURBANCE OF THE CONNECTIVE TISSUE GROUND SUBSTANCE

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THE BOUNDARY zone between epithelium and connective tissue in the skin and in other structures is formed by an optically homogeneous substance, which, together with the embedded fibers, has been termed the "basement membrane."[1] The major component of the homogeneous ground substance is an insoluble carbohydrate-protein complex which is thought to be highly polymerized. Although relatively resistant to chemical treatment, it may exhibit lability in certain physiologic and pathologic processes.

The ground substance of the basement membrane and of the subjacent connective tissue may be demonstrated in sections of gingival tissue with the periodic acid-leucofuchsin method.[2] Since many of the disturbances of the gingiva originate in the connective tissue this procedure has been very useful in evaluating these changes.

In desquamative gingivitis one sees extreme changes in the gingiva, suggesting that the connective tissue is altered. It is observed, clinically, that the slightest pressure of the finger or from an air blast causes a clean separation of the epithelial layer from the underlying connective tissue in an almost spontaneous manner. The gingiva are marked by many ulcerated bleeding areas.

The histopathologic changes in this disturbance were studied by Ziskin and Zegarelli.[3] They reported degeneration of the epithelium and edema and inflammation in the connective tissue layer. We have been interested in some of the intimate mechanisms which lie behind these changes.

Observations made in the clinic, interpreted in the light of the newer ideas about the ground substance, suggested an explanation. The interpretation is based on studies of gingival biopsies fixed by the freezing-drying method and treated by specific histochemical procedures. The prominent basement membrane shown to be present in normal gingiva was absent in desquamative gingivitis. A further deviation from the normal was the greatly increased quantity of carbohydrate-containing substance soluble in water and insoluble in alcohol. As these water-soluble components may arise from the action of depolymerizing enzymes, it was deduced that the connective tissues of the gingiva in desquamative gingivitis contain abnormal quantities of such activities. It has been thought that a low estrogen level might lie behind the symptoms in desquamative gingivitis.[3] Such a proposal could be supported by the hypothesis of Gersh and Catchpole[1] that the enzymatic activity of the connective tissue is subject to hormonal influences.

Received for publication, December 2, 1949.

MATERIAL AND METHOD

Eight patients who suffered from desquamative gingivitis were studied.* (Table I.) Six of the group were women. With the exception of one who was observed during a period of remission all had exfoliative lesions of the gingiva. Two of the patients also had skin lesions which were diagnosed as lichen planus. Persons of varying age with normal gingival tissues were also studied as controls.

TABLE I

SUMMARY OF PATIENTS

NUMBER	AGE (YRS.)	SEX	CLINICAL REMARKS
1	45	F	Acute desquamative lesions of gingiva with periods of remission. History of breast "cyst" which was removed.
2	55	F	History of desquamative gingivitis of approximately 10 years' duration. Patient in period of remission at time of biopsy. Tissues appeared red and rough. Also past diagnosis of lichen planus.
3	57	F	Acute desquamative lesions. Also history of secondary anemia.
4	72	F	Desquamative lesions over a period of approximately 20 years.
5	28	F	Splenectomy 2 years ago for treatment of thrombocytopenic purpura. Blood picture now normal. Acute lesions on gingiva and on cheeks for the past year. Patient also has lesions of skin which have been diagnosed as lichen planus.
6	44	F	Desquamative lesions limited to gingiva, edentulous areas free. Lesions reappeared 3 months after gingivectomy. Patient observed for 2 years. Carcinoma of breast developed during second year. Patient died 6 months after operation.
7	40	M	Acute desquamative lesions, limited, for a period of 1½ years. Tissues very fragile.
8	47	M	Acute desquamative lesions for the past year.

In almost all cases the biopsies were fixed by freezing and drying. A few specimens were fixed in 10 per cent formalin or Zenker's solution for comparison and for morphologic study. The following routine was used: Sections of gingival tissue were removed, without anesthesia where possible, and were immediately frozen in isopentane which had been chilled to approximately −150° C. The tissues were then dehydrated at about −30° C. in vacuo. The soluble and diffusible components are retained by this procedure, the proteins are largely undenatured and enzymatic activity is stopped. The tissue was then infiltrated with paraffin (m.p. 56-58° C.) in vacuo and embedded. Sections were cut at 4 μ for cytologic purposes and at 10 μ for solubility studies. They were mounted on albuminized slides with slight finger pressure. The paraffin was then removed with petroleum ether. The polysaccharides and polysaccharide-protein complexes were then demonstrated by staining with the Hotchkiss leucofuchsin-periodic acid method.[2] The staining procedure was

*We wish to thank Dr. E. Ball of Cincinnati and Dr. L. Sreebny of the University of Illinois Research Hospital Dental Clinic who provided some of the clinical material.

modified to yield information on the solubility of the ground substance, by treating neighboring sections before staining in the following ways: (1) overnight denaturation in ethanol; (2) treatment for one hour with M/15 phosphate buffer (pH 7) followed by alcohol precipitation; and (3) alcohol precipitation followed by treatment with buffer.

When the sections were treated with buffer the following routine was used: The deparaffinized slide was placed in a Petri dish with some moist toweling. The section was covered with 5 drops of buffer and after one hour the solution was decanted and the section washed with 5 drops of the buffer. The slide was then immersed in absolute alcohol overnight.

As compared with the depth of staining after Procedure 1, the other steps always yielded a less intense color. The staining was interpreted as follows: The removal of soluble material from the tissue was shown by a diminished intensity in the red staining with the periodic acid-leucofuchsin reagents. Treatment of the preparation with water (phosphate buffer) followed by alcohol denaturation probably removed water-soluble glycoproteins and some of the smaller molecules such as glycopolypeptides and uronic acids. However, when the tissues were first denatured by overnight immersion in absolute alcohol these substances were precipitated. Glycoproteins were irreversibly precipitated, while the residues of glycoprotein such as glycopolypeptides and uronic acids became water soluble again upon treatment with buffer. These two methods of preparations, that is, buffer extraction followed by alcohol denaturation and alcohol denaturation followed by buffer were compared with a control which was stained following alcohol denaturation only. Comparison of the staining intensity of the sections treated in these ways was noted on a 1 to 4 plus scale. These results are summarized in Table III.

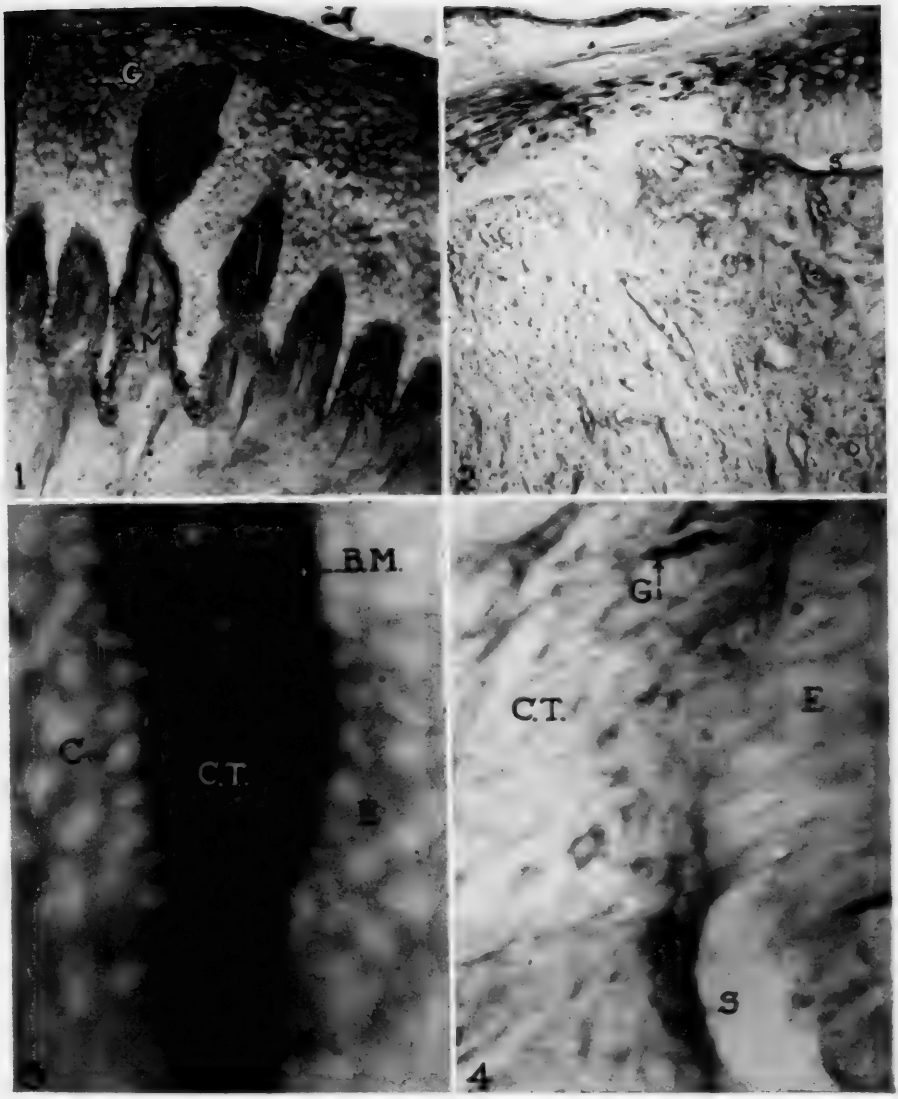
Some of the morphologic characteristics were noted in sections which were counterstained with malachite green and in others stained with hematoxylin and eosin.

OBSERVATIONS

Normal Gingiva

The ground substance of the connective tissue in normal gingival sections denatured in absolute alcohol prior to staining was colored intensely red with the periodic acid-leucofuchsin method (Figs. 1 and 3). The fibers were sometimes masked by the omnipresent ground substance; their staining may be attributed either to a glycoprotein coating or to an intrinsic glycoprotein structure. The basement membrane appeared as a brilliant red undulating line of varying thickness. Fibroblasts were the predominant cells seen in the connective tissue. Their nuclei were large and the cytoplasm only infrequently contained glycogen or glycoprotein granules.

The epithelial cells were separated by a cement substance which also stained red and gave the appearance of a fine network (Figs. 1 and 3). Many of the cells, particularly those of the middle third of the epithelial layer, contained carbohydrate material in the cytoplasm which could be removed by incubation in saliva, and which was presumably glycogen. (Fig. 1.)



Figs. 1-8.—All photomicrographs made from tissues fixed by freezing-drying and stained by the periodic acid-leucofuchsin method. The sections were denatured in absolute alcohol for 16 hours before staining except where other preliminary treatment is described.

Key: *B*, blood vessel; *B.M.*, basement membrane; *C*, cement substance; *C.T.*, connective tissue; *E*, epithelium; *G* glycogen; *I*, intercellular space; *L*, leucocyte; *N*, nucleus; *S*, separation at connective tissue-epithelial junction.

Fig. 1.—Section of normal gingival tissue. Ground substance is almost homogeneously stained. Basement membrane is definitely outlined about some papillae; plane of sectioning prevents its demonstration throughout section. ×165 (orig. mag.).

Fig. 2.—Section of gingival tissue from patient with desquamative gingivitis. Some components of ground substance are no longer present. Basement membrane is absent or very attenuated. At one site (*S*), epithelium has separated from connective tissue at their boundary. Connective tissue papillae are virtually absent and epithelial layer is abnormally thin. ×165 (orig. mag.).

Fig. 3.—Basement membrane appears as heavily stained wavy line in this normal preparation. Connective tissue is rather homogeneously stained and stroma is seen only indistinctly. Glycoprotein cementing substance of epithelial cells creates a network effect. ×980 (orig. mag.).

Fig. 4.—In desquamative gingivitis the basement membrane separating connective tissue from epithelium is dissolved, hence there is no sharp demarcation. Ground substance is largely gone and stroma is revealed. Many of the connective tissue fibers are swollen. Note separation of connective tissue and epithelial elements at *S*. A connective tissue cell containing glycogen may be seen near upper margin. ×1000 (orig. mag.).

When sections of normal gingiva were treated with phosphate buffer (pH 7) for one hour, then denatured with ethanol and stained, the ground substance did not stain as intensely as in untreated preparations. This indicates that some water-soluble components may have been present. When the sections were first denatured in alcohol and then treated with buffer the amount of material extracted was considerably less, indicating the presence of small amounts of glycoprotein residues or uronic acid derivatives in unextracted sections.

Gingiva in Desquamative gingivitis

In the tissues obtained from patients with desquamative gingivitis, the ground substance in ethanol-denatured sections stained in varying degree depending upon the severity of the affection. In all cases the intensity of redness was greatly reduced as compared to normal tissues (Table II). In some instances the ground substance was almost entirely absent (Figs. 2 and 4). Sometimes the connective tissue fibers were seen more distinctly having been unmasked by the loss of the ground substance. The basement membrane was absent or weakly stained in many areas; in others it had swelled or was discontinuous. The basement membranes of the many dilated blood vessels in the connective tissue were similarly affected (Fig. 6). The connective tissue fibers in some areas showed various degrees of disintegration.

TABLE II

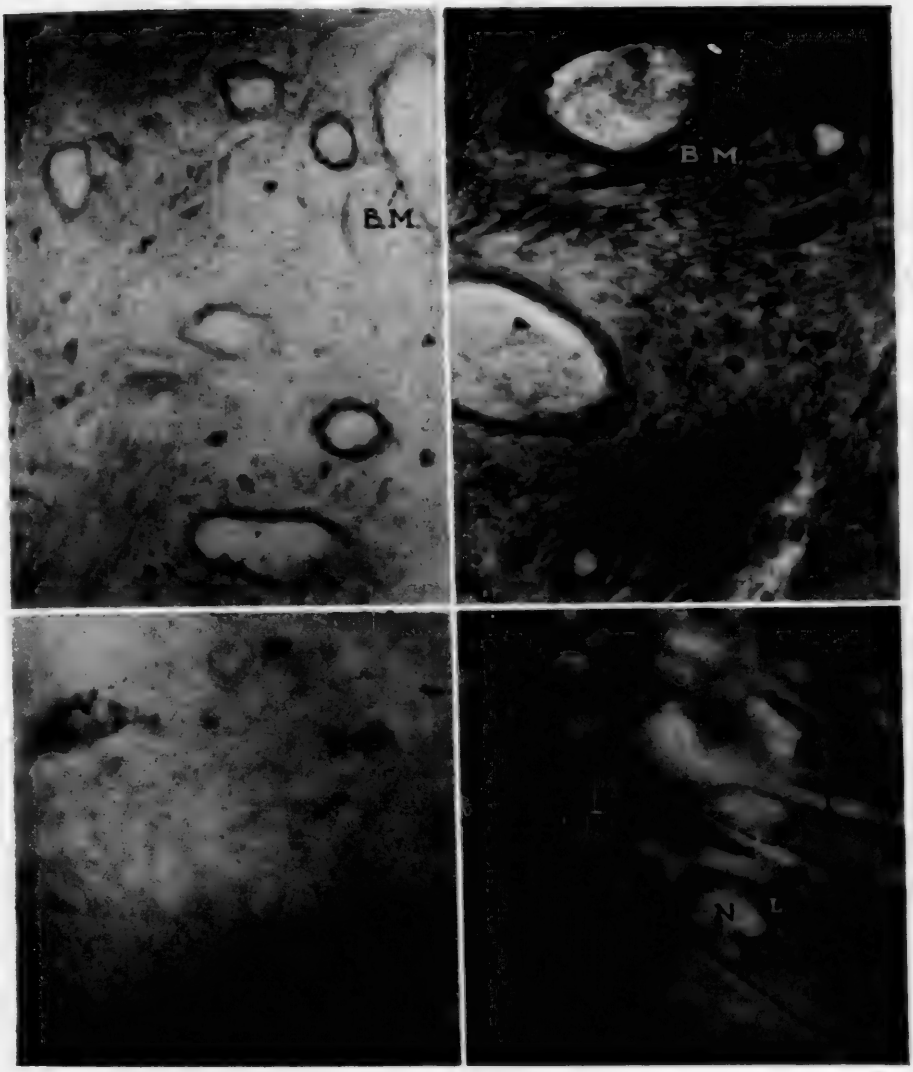
DESCRIPTION OF STAINING REACTION WITH PERIODIC ACID-LEUCOFUCHSIN METHOD OF CERTAIN TISSUE COMPONENTS IN NORMAL GINGIVA AND IN DESQUAMATIVE GINGIVITIS

	NORMAL	DESQUAMATIVE GINGIVITIS
Basement membrane	Sharply defined undulating line	Absent or discontinuous, sometimes swollen
Ground substance of subepithelial connective tissue	Intense red	Diffuse pink
Cement substance of epithelial cells	Well-defined light pink lines	Absent or swollen and disorganized

The tissue was infiltrated with dense accumulations of lymphocytes, polymorphonuclear leucocytes, plasma cells, and macrophages. Some of the fibroblasts contained red staining granules which were removed after incubating the sections with saliva and are therefore presumed to be glycogen. Some of the macrophages were filled with granules and aggregates of glycoprotein which may be phagocytized residues of ground substance (Fig. 7). Occasionally masses of microorganisms were seen in areas where the epithelium was gone.

The epithelial cells, particularly those of the basal layer, differed from the normal. The glycoprotein cement substance appeared swollen or had dissolved, leaving spaces between the cells (Fig. 8). This was particularly seen about those cells adjacent to the regions of most marked change in the connective tissue.

Those tissues which had been fixed in formalin prior to histochemical study showed changes similar to those already described, but the staining of



Figs. 5 and 6.—Sections through connective tissue of biopsy specimen. This was included to show presence of alcohol-insoluble, water-soluble material in desquamative gingivitis as demonstrated by differences in staining intensity. Clear areas of print were reproduced to same density so that prints are comparable. ×320 (orig. mag.).

Section in Fig. 5 was alcohol denatured 16 hours, then treated with phosphate buffer (pH. 7). Staining reaction of ground substance and basement membrane about blood vessels is considerably attenuated when compared with Fig. 6.

The section in Fig. 6 was alcohol denatured 16 hours. Note dilated blood vessels surrounded by swollen basement membrane. One of these vessels has basement membrane which is in more advanced state of disorganization.

Fig. 7.—Glycogen was removed from this section by incubating with saliva after alcohol precipitation of proteins. Connective tissue cells containing granules of glycoprotein are shown. ×1000 (orig. mag.).

Fig. 8.—Epithelial cells with broad intercellular spaces produced by solution of glycoprotein-cementing substance. Several cells are outlined by interrupted lines. Compare with Figs. 1 and 3. ×1250 (orig. mag.).

the ground substance was diffuse, probably because some water-soluble components had dissolved and some of the proteins had swelled during the fixation.

Extraction of the frozen-dried sections with buffer showed marked qualitative differences from the normal gingiva (Figs. 5 and 6). The decrement in the intensity of the staining after extracting at pH 7 followed by alcohol denaturation was greater than that observed in the normal tissues. This decrease in staining when compared with the normal tissues was also greater when the preparations were denatured with ethanol and then extracted with buffer (Table III). These differences were ascribed to the presence of large quantities of polysaccharides and glycoprotein residues which were soluble in water but insoluble in alcohol. Since some of these substances are extracted and others irreversibly denatured during immersion in formalin, tissues fixed in that manner could not be studied for solubility changes.

TABLE III

COMPARISON OF SOLUBILITY OF GROUND SUBSTANCE IN NORMAL GINGIVA AND IN DESQUAMATIVE GINGIVITIS

REAGENTS	NORMAL	DESQUAMATIVE GINGIVITIS
Ethanol	++++	++
M/15 phosphate buffer pH 7 followed by ethanol	++	±
Ethanol followed by M/15 phosphate Buffer pH 7	+++	+

Relative intensity of staining reaction with the Hotchkiss procedure indicated by + to ++++.

DISCUSSION

The ground substance of adult connective tissue is thought to be organized as a highly polymerized carbohydrate-protein complex[1] and, as is characteristic of such aggregations, it is not readily soluble in water or in alcohol. Some water-soluble glycoproteins and residues of glycoprotein molecules may be present. These are insoluble in alcohol. The glycoprotein residues, glycopolypeptides and uronic acid derivatives, are, however, not irreversibly precipitated by alcohol and they can be redissolved in water. These latter components ordinarily seem to be present only in relatively small quantities. It is probable that these various fractions, and some others which we have not identified, are in equilibrium with each other. It is attractive to consider that the equilibrium is in part mediated by a group of enzymes having to do with the synthesis and depolymerization of carbohydrate-protein complexes. As the ground substance is labile there may be shifts in the relative quantities of the fractions as, for instance, an increase in the amount of substance which is soluble in water but insoluble in alcohol. This may come about when the larger glycoprotein molecules, which are ordinarily not soluble in water, are depolymerized and fragmented. Such shifts in the relative amounts of the various components of the ground substance were previously noted in a num-

ber of conditions as for example in rapidly growing tumors and in inflammation,[1] in the stimulated ovary,[4] and in the relaxed pubic symphysis of the guinea pig.[5]

Sections containing adult connective tissues which are treated with water following alcohol precipitation show an increased intensity in staining by the Hotchkiss method.[1] This may be due to swelling (depolymerization) of the denatured glycoprotein and greater availability of reacting groups. On the other hand it has been noted above that similar preparations of normal gingiva show some decrement in staining intensity (Table III). This difference may be explained when one considers that the gingiva are exposed to constant trauma and to pathogenic microorganisms and that a state of very mild inflammation or injury response is therefore "normal." In such a state, then, some elements of the ground substance are depolymerized. These give rise to the fraction which is soluble in water and insoluble in alcohol.

In desquamative gingivitis, alteration in the stainability of the basement membrane or its absence are indications of the depolymerization of the highly polymerized components of the ground substance. This is further reflected by the considerable increase in water-soluble, alcohol-insoluble materials and by the markedly changed appearance of the ground substance. The epithelium peels off readily because the cementing substance of the cells and the cementing function of the basement membrane and ground substance have been lost, reduced in amount, or become altered.

In vitro studies have shown that certain elements of the connective tissues can be attacked by a number of enzymes. The effect of a "collagenase" found in *Clostridium welchii* filtrates was described by Robb-Smith[6] in 1945. Burnet and Stone[7] attributed the desquamation of the intestinal epithelial cells and the solution of the intercellular cement substance in *Vibrio cholerae* infections to a group of "mucinases" which are present in *V. cholerae* filtrates. These mucinases are believed to be related to the collagenase of *Cl. welchii toxin*. The specific effects of collagenase on connective tissue ground substance and basement membrane were demonstrated by Gersh and Catchpole[1] who then went on to develop the idea that similar enzymes are liberated by the connective tissue cells under local and hormonal influences.

From the evidence presented, it sems that the tissues in desquamative gingivitis have abnormal quantities of collagenase-like factors. Such activity could explain the solution of ground substance, the basement membrane, and the glycoprotein-cementing substance of the epithelial cells of the basal layers. While we have not yet identified or demonstrated the enzymic factors in the lesion of desquamative gingivitis, some preliminary experiments support the view that such substances are present.

The fact that two of our patients had lesions of the breast (carcinoma and cyst, respectively) and that two had other skin lesions diagnosed as lichen planus makes it appear that the gingival lesions are local manifestations of a systemic disorder.

The predominance of female patients in our series is a typical distribution which has also been noted by others. This has led to the assumption that the sex hormones are implicated in some way[3] and patients with desquamative

and other lesions of the gingiva are reported to have been treated successfully with Progynon, locally and parenterally.[3, 8, 9] There is considerable evidence that the sex hormones, topically applied, can exert local effects.[10] However, a definite sequence of relationships has not been established in desquamative gingivitis and the estrogen level in these cases has not been investigated adequately.

The gingiva and oral mucous membranes are influenced by the sex hormones. During menstruation and pregnancy it is not uncommon for the gingival tissues to become swollen and edematous. The severe alteration of the ground substance in desquamative gingivitis may involve an extension of the same process in which the connective tissue cells have a heightened reactivity to changes in sex hormone level. They may then react by secreting certain components of the ground substance as well as by elaboration of collagenase-like enzymes.

CONCLUSIONS

1. In desquamative gingivitis there is a disturbance of the ground substance of the gingival connective tissue. This is manifested by changes in the properties of the interstitial components: (a) The basement membrane is either absent or partially dissolved. (b) The ground substance contains increased quantities of water-soluble glycoprotein and water-soluble alcohol-insoluble, glycoprotein residues.

2. It is proposed that these changes are due in part to the production of abnormal amounts of depolymerizing enzymes which affect the ground substance and the cementing substance of the epithelial cells in the manner described.

3. The possible influence of the female sex hormones in inducing the changes of the ground substance is considered.

We are indebted to Dr. Granville Bennett who extended the facilities of his laboratory. We are pleased to thank Dr. I. Gersh and Dr. H. R. Catchpole for their counsel.

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STUDIES IN THE REGENERATION AND REATTACHMENT OF SUPPORTING STRUCTURES OF THE TEETH

I. Soft Tissue Reattachment

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INTRODUCTION

A SURVEY of the literature reveals a sharp cleavage of opinion on the possibility of functional regeneration and reattachment of the supporting tissues of the teeth after these tissues have been extensively damaged or lost during the course of periodontal disease. This subject has been frequently discussed in the literature. Excellent reviews are to be found in Goldman's book,[1] and more recently in papers by Orban[2] and Morris.[3]

The experimental work in the field has been mainly of two types. In the first type, studies have been made of repair following the destruction of sections of the supporting structures remote from and not involving the gingival crevice.[4, 5] Since the gingival crevice may be important in this problem it has been questioned whether the results of such work may be accepted as evidence of the possibility of regeneration and attachment following pocket formation in periodontal disease. The second type has been the study of repair following the creation of artificial pockets produced by surgical separation of the soft tissues from the tooth.[6] In this study Skillen and Lundquist found that only little connective tissue reattachment occurred. Their conclusion was that the epithelium seemed to proliferate and cover the denuded tissue so rapidly that all further connective tissue reattachment was prevented.

As the various reviews emphasize, there are many complicating factors in the clinical periodontal problem of the regeneration and reattachment of the supporting structures of the teeth. It seemed advisable, therefore, to study the situation under less complex conditions, i.e., by bringing about the loss of supporting structure by surgical means in healthy tissue. While it is recognized that this experimental situation differs in some respects from the clinical, it appeared more favorable for the investigation of some of the fundamental aspects of this problem, i.e., the potentiality of the supporting structures for regeneration and reattachment and the study of the underlying factors and principles involved in the process.

This paper deals with a study of the reattachment of soft tissues to the tooth. Connective tissue reattachment of the soft tissues to the tooth by means of a deposition of new cementum has been repeatedly secured in the present investigation.*

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*A report on the regeneration of alveolar bone will be made later.

PROCEDURE

The experimental animals were dogs with healthy gingival tissues. Only the four canine teeth were used. By means of a disc stone, a groove was cut into the tooth approximately 1 mm. from the gingival margin (Fig. 3). This groove served as a point of reference from which the level of the gingival sulcus or pocket could be measured. Thus it was possible to obtain accurate data on the gingival recession and of reattachment of the surgical flap. A gingival flap was made exposing the overlying alveolar bone, care being taken to detach the complete periosteum including the inner or cambium layer. Then a section of bone and periodontal membrane approximately 7 × 6 mm. was removed exposing the tooth. The flap thus detached measured approximately 9 mm. from the bottom of the gingival sulcus, or 12 mm. from the groove in the tooth. In some cases the tooth was scaled; in others, no scaling was done. The flap was sutured into place. Following surgery, in the greater apical part of the flap the disposition of the tissues from within outwards was as follows: denuded or scaled tooth surface, inner layer of periosteum, outer layer of periosteum, connective tissue of the gingiva, oral epithelium. Figs. 1 and 2 are sketches illustrating the disposition of the tissues before and after the surgical procedure.

By means of a probe graduated in millimeters, the level of the bottom of the gingival sulcus was measured from the gingival mark on the tooth before operation and again at the end of the healing period. Nineteen upper and seven lower teeth were used in this phase of the study. The animals were sacrificed at various intervals from 5 days to 1 year and blocks were removed for sectioning. Paraffin sections were made after fixation in 10 per cent formol-saline and decalcification in 5 per cent formol-nitric acid, and were stained with hematoxylin and eosin. The oldest animal described in this paper was sacrificed 49 days after operation.

OBSERVATIONS ON THE LIVING ANIMAL

In the upper teeth when measured by a probe, 6 to 8 mm. of reattachment appeared to have occurred in all but one instance, when the healing period exceeded 18 days. Before that period had elapsed, sufficient pressure could not be exerted on the probe to determine whether attachment had taken place, without the risk of tearing the healing wound. After the eighteenth day the wound appeared to have healed and the gingiva had resumed a normal appearance (Fig. 3). Usually 1 to 3 mm. of recession were found.

On the other hand, in the lower canines, only 1 to 3 mm. of attachment appears to have occurred. Often there was considerable recession over the area, the pocket depth being related to the amount of recession. The gingiva did not regain the normal appearance as did the gingiva in the maxilla.

HISTOLOGICAL OBSERVATIONS

The histological examination of the specimens permitted the differentiation of two groups of cases. One, represented almost solely in the lower jaw, ended with little reattachment to the tooth and with the formation of a true epi-

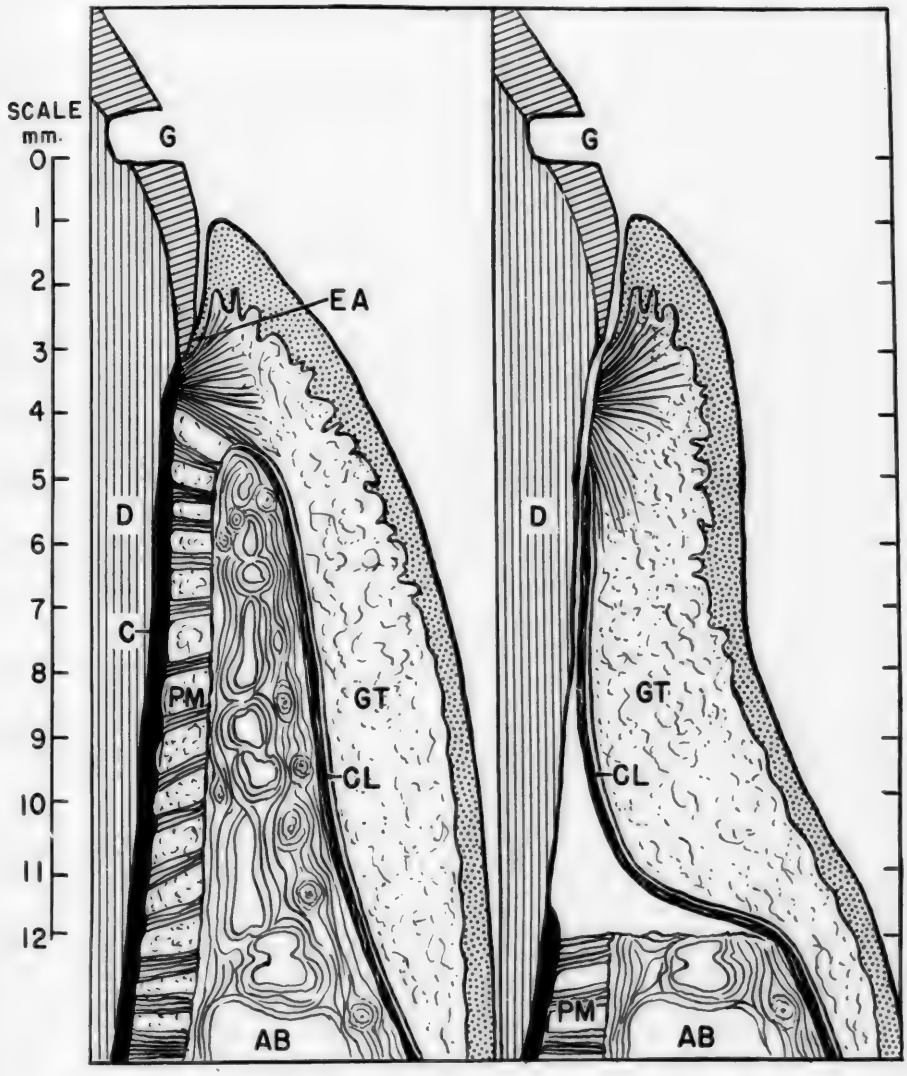

Fig. 1. Fig. 2.

Figs. 1 to 14.—Key to abbreviations in illustrations: *A* = artefact (separation believed to have occurred during the preparation of the sections); *AB* = alveolar bone; *B* = base of pocket; *C* = cementum; *CB* = cementoblast; *CC* = cementocyte; *CE* = crevice epithelium; *CI* = cellular infiltration; *CL* = cambium layer; *CTF* = Connective tissue fibers; *D* = dentin; *E* = epithelium; *EA* = epithelial attachment; *G* = groove in tooth; *GT* = gingival tissues; *IA* = inactive area described in text; *J* = junction of old and new attachments; *NAT* = newly attached tissue; *NC* = new cementum; *OC* = old cementum; *OD* = old dentin; *P* = polymorphonuclear leucocyte; *PM* = periodontal membrane; *R* = resportion; *RL* = resorbed dentin with reversed line; *RR* = resorbed and reattached area; *SA* = separation artefact; and *X* = insert.

Figs. 1 and 2.—Illustrations of the disposition of the supporting structures of the teeth before and after surgical procedure.

thelialized pocket. In addition, gingival recession occurred in some of the teeth. The second group, those in the upper jaw, can be considered as successful connective tissue attachment of the detached gingiva to the exposed tooth surface. Some regeneration of the removed alveolar bone could be observed in most of the experimental animals where attachment of the flap occurred.

Fig. 4 is a photomicrograph of a lower canine with a 14-day healing period. No cementum is apparent on the surface of the tooth from which the supporting tissues have been detached; also, there is some loss of dentin. The possibility must be considered that at least part of this lost tooth structure may have been removed at operation together with the bone and periodontal membrane or by scaling. The pocket is inadequately covered by epithelium, little being present over the lower half of the pocket. However, what appears to be an epithelial attachment (high-power insert) was observed in a field about 1 mm. from the base of the artificial pocket which was approximately 10 mm. in depth. The connective tissue is inflamed, with an increased cellular infiltration around some hairs which were found in many of the pockets in the lower jaw. In contrast, only a few hairs were found in the maxillary gingival cases on microscopic examination. The presence of these hairs in the tissue will be discussed later in this paper.

In the upper jaw, connective tissue attachment of the flap to the tooth, by means of the deposition of new cementum, occurred over approximately ¾ of the denuded tooth surface.

Fig. 5 is a photomicrograph of an upper canine showing attachment after a 49-day healing period. Figs. 6, 7, and 8 are higher magnifications of selected fields from Fig. 5. Fig. 6 shows the junction of the old and new attachments. Above the junction, in the zone of new attachment, all of the cementum and some of the dentin have been lost. New cellular cementum has been laid down on the dentin but has been pulled away. This separation is considered to be artefact produced by post-mortem procedures (see description of Fig. 7). The connective tissue fibers appear to be attached in the usual manner but run in a direction parallel to the tooth rather than in the characteristic oblique or horizontal direction. This was the arrangement of the connective tissue fibers wherever the reattachment had taken place. Note the absence of any appreciable cellular infiltration.

Fig. 7 is a view of the same section about ½ mm. gingivally to that of Fig. 6. Both attached cementum and an extension of the artificially detached cementum shown in Fig. 6 can be seen. In this photograph it is observed that at least some of the loss of tooth structure is due to resorption. Note the well-differentiated cementoblast layer (insert is higher magnification at point *x*). In this section the parallel arrangement of the connective tissue fibers described above is quite distinct.

Fig. 8 is a view of the gingival sulcus of the specimen shown in Fig. 5. It is evident that some dentin had been resorbed but so far much less cemental apposition has occurred than was shown in Figs. 6 and 7. Note that the epithelium had not proliferated rootwise as was the case with the lowers. There is little cellular infiltration.

Fig. 3. Fig. 4.

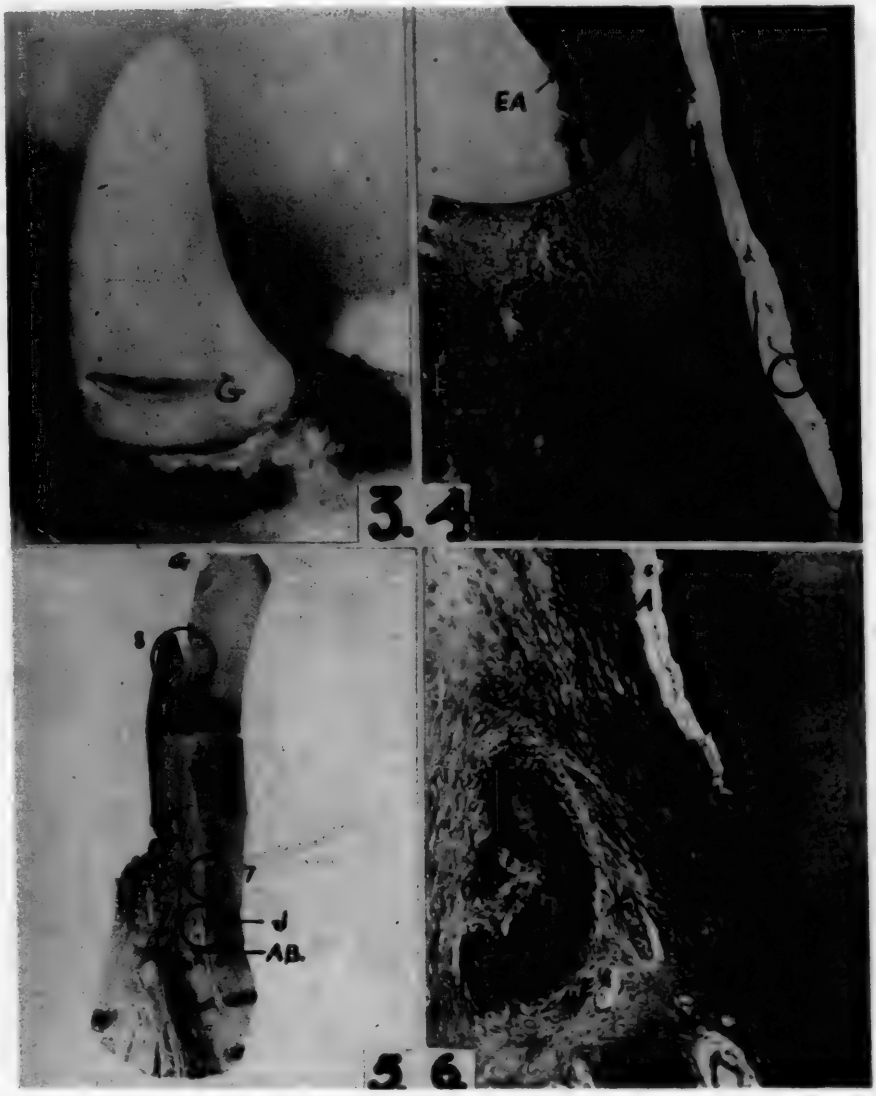

Fig. 5. Fig. 6.

Fig. 3.—Twenty-two-day specimen, upper canine. Typical of appearance of gingiva.

Fig. 4.—Fourteen-day specimen, lower canine where reattachment failed. Note absence of cementum and loss of some dentin.

Fig. 5.—Forty-nine-day specimen, upper canine, showing reattachment.

Fig. 6.—Forty-nine-day specimen, higher magnification of Fig. 5 at the junction of old and new attachments. (Area enclosed in circle at *J*, Fig. 5.)

Sections of all cases up to the nineteenth day show a separation of the soft tissues from the tooth. This is believed to be artefact, caused either in the trimming of the block or during the preparation of the sections, the early union between the teeth and soft tissues being weak. From the nineteenth to the thirty-fifth day of healing, many such artefacts were encountered but evidence of reattachment was frequently found which suggested that in the living animal the tissues had been attached. This is illustrated in Figs. 9 and 10. Fig. 9 is a photomicrograph of a field of a section from an upper canine, 19-day healing period, in which it is apparent that some of the connective tissue fibers are attached even though in most of the section the soft tissues were separated from the tooth. This specimen shows the loss of cementum and some dentin from the tooth and the parallel arrangement of the connective tissue fibers, described in previous slides. Some cellular infiltration is still present. Fig. 10, 21-day healing period, illustrates a type of separation that is often encountered. The attached tags of tissue suggest that the connective tissue was torn. This view also shows early evidence of cemental apposition on resorbed dentin. However, in Fig. 11, which shows a field of another section from the same tooth, there is a considerable thickness of new cellular cementum laid down on resorbed dentin. It is evident that activity in resorption and apposition varies in different areas along the tooth. Indeed, in some of the specimens where attachment took place, areas were noted where little if any evidence of resorption or apposition was apparent (Fig. 8). Again, other areas were seen where the tissues were separated from the tooth, in which little if any resorption, apposition, or other evidences of reattachment could be found. Fig. 12, a view of an upper canine, 49-day healing period, shows such an area. The resorbed and attached area (toward the top of the photomicrograph) lies gingivally to the area described. However, under high power, cells were observed which appeared to have differentiated into cementoblasts. They were rounded up, deeper stained, and were related to a heavier staining intercellular substance. They appeared much more widely scattered than the cementoblasts shown in Fig. 7. If these cells are cementoblasts, attachment probably would have occurred later, if it had not already done so. The lack of cellular infiltration suggests that, even if attachment has not taken place, the area does not communicate with the gingival crevice.

One of the most interesting findings in this study is the behavior of the epithelium. Fig. 13 is a view of the crevice epithelium and adjacent gingiva of the upper canine, twenty-second day of healing, where on microscopic examination the soft tissues were found to be separated from the tooth. We have stated that such separations are believed to be artefact. The fact that the epithelium did not proliferate rootwise in this specimen suggests that in the living animal attachment had taken place. Also of interest is the relative lack of cellular infiltration into the connective tissue of the gingiva.

What appears to be a regeneration of alveolar bone was observed in many of the sections where attachment took place. Fig. 14, a view of an upper canine, 22-day healing period, is a typical example. The characteristic loss of cementum

Fig. 7. Fig. 8.

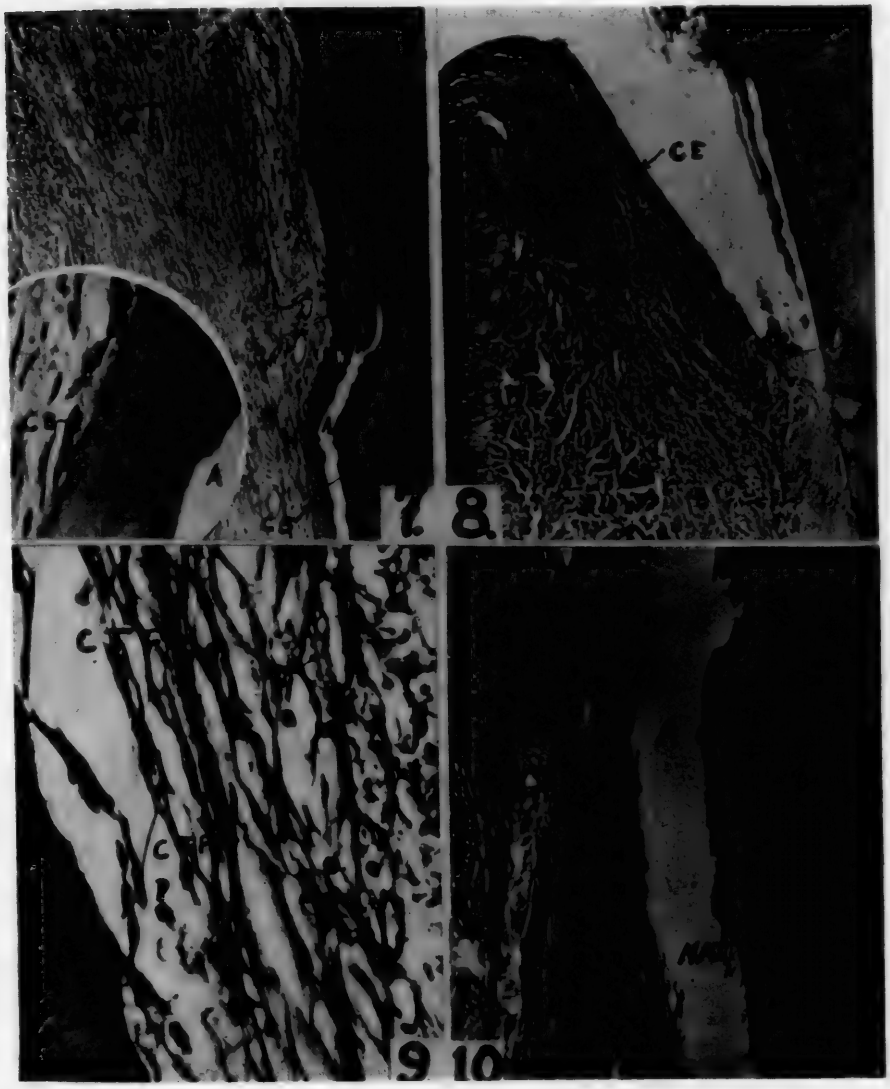

Fig. 9. Fig. 10.

Fig. 7.—Forty-nine-day specimen, higher magnification of Fig. 5, one-half millimeter gingivally to Fig. 6. (Area enclosed in circle at 7, Fig. 5.)

Fig. 8.—Forty-nine-day specimen, higher magnification of Fig. 5 showing gingival sulcus. (Area enclosed in circle at 8, Fig. 5.)

Fig. 9.—Nineteen-day specimen, upper canine. Note loss of cementum and some dentin, and the parallel arrangement of new connective tissue fibers.

Fig. 10.—Twenty-one-day specimen, upper canine.

and some dentin, apposition of new cementum, parallel direction of the fibers, and the newly differentiated cementoblasts can be seen. The space between the new cementum and the dentin is believed to be artefact.

DISCUSSION

Although the technic for detachment of the periosteum from the alveolar bone and its replacement over the denuded tooth surface was developed because of the alleged osteogenic potency of the periosteum and its property of acting as a limited membrane, the results of the experiment do not preclude a different interpretation. It is possible that the loose connective tissue of the cambium layer is to be preferred to the dense connective tissue of the gingiva for reattachment. This does not necessarily mean that under suitable conditions the required cellular differentiation for attachment cannot take place when healing occurs by granulation tissue arising from the connective tissue of the gingiva. Also in comparing our results with those reported by Skillen and Lundquist,[6] the possibility must be considered that better adaptation of the detached tissues to the tooth may be a factor in the end result.

The result in the mandible, where the prominent feature was the development of a true epithelialized pocket rather than connective tissue attachment, appears similar to the result reported by Skillen and Lundquist. A number of possibilities for failure of attachment have been considered. First it was noted that the periosteum was more difficult to remove from the alveolar bone in the lower canines than in the upper canines. Sections of alveolar bone removed after the periosteum had been detached showed that considerably more of the cambium layer remained attached to the bone in the lower than was the case with the upper jaw where little soft tissue could be observed remaining on the bone. Another possible reason for failure was that in many cases hairs were found in the wounds in the lowers, possibly because the dogs rubbed the gingiva with their paws after operation. Only very occasionally was hair seen in the uppers. Another factor might be the higher muscle attachment. Obviously these questions and others brought up in the discussion can only be answered by further work, some of which is now under way.

The variation in activity, indicated by the decided difference in the amount of resorption and apposition along the tooth, where reattachment had taken place, is difficult to explain in the light of present knowledge. One would not expect an even resorption but the reason for the variation in the amounts of cemental apposition in different positions on the tooth is not understood. In general, the greatest thickness of new cementum was found toward the base of the wound.

In commenting on the behavior of the epithelium all that can be stated is that it seemed to proliferate apically when the connective tissue attachment failed but not when it took place. Thus it cannot be argued that attachment was prevented by the downgrowth of epithelium. It appears that failure of attachment permitted the proliferation of the epithelium.

In most cases where reattachment had taken place some regeneration of alveolar bone was noted on histological examination. However, in a number of

older experimental cases where reattachment of the soft tissues to the tooth appeared in the gross to have occurred, a second gingival flap was made for the purpose of examining the alveolar bone. It was found that there was some

Fig. 11. Fig. 12.

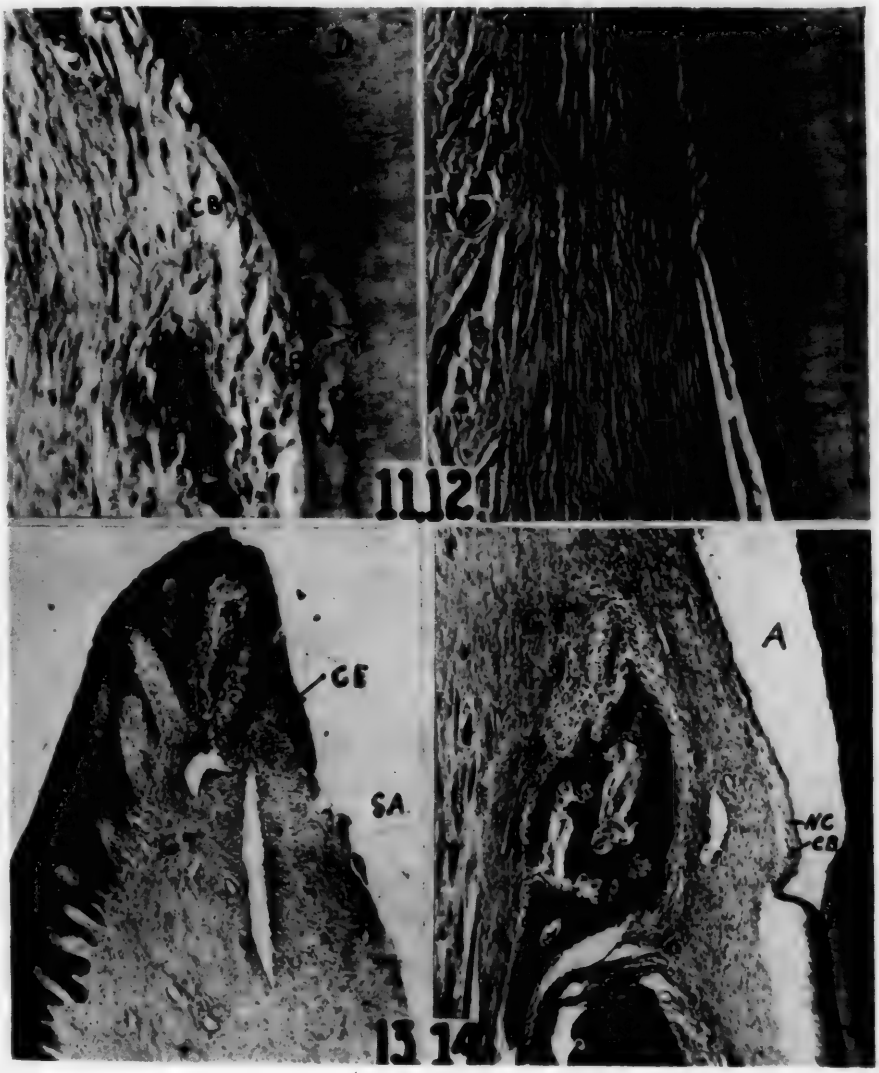

Fig. 13. Fig. 14.

Fig. 11.—Twenty-one-day specimen, upper canine.

Fig. 12.—Forty-nine-day specimen, upper canine.

Fig. 13.—Twenty-two-day specimen, upper canine. The separation artefact was so wide that the tooth could not be shown in the same field at this magnification. Note the relative lack of cellular infiltration.

Fig. 14.—Twenty-two-day specimen, upper canine.

slight regeneration of the alveolar bone at the base of the wound. However, resorption of the bone at the edges of the wound was noted. Appreciable bone regeneration had not occurred up to one year after operation when examined in the living animal.

The reason only seven lower canines as against nineteen maxillary canines were used in this study was that preliminary studies had already indicated that the upper canines were to be preferred over the lowers for an attachment study. It is obvious, however, that we have much to learn from negative as well as positive results.

CONCLUSIONS

1. In dogs, following the surgical destruction of a section of the periodontal membrane and alveolar bone, connective tissue reattachment of the gingival tissues to the tooth by a deposition of new cementum was repeatedly obtained.

2. Uneven resorption of both cementum and dentin may take place prior to cemental apposition.

3. New cementum may be laid down on the old cementum or directly on dentin.

4. In the reattached gingiva, the connective tissue fibers run in a direction parallel to the tooth rather than in the characteristic oblique or horizontal direction.

5. The oral epithelium appears to proliferate downward when reattachment fails but not when it takes place.

6. Some regeneration of alveolar bone occurred.

The histological sections were prepared by Mr. D. H. Beaton; the photomicrographs by Dr. W. Stanley Hartroft.

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THE OXYGEN CONSUMPTION OF HEALING GINGIVA

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ALTHOUGH the oxygen consumption in the course of healing has been determined for several tissues[1-4] there has been no comparable study of gingiva. Up to now, microscopic investigation[5-7] has been the only source of information regarding the details of gingival repair following gingivectomy. The demonstration of the applicability of the Warburg technic[8] for the determination of the oxygen consumption of normal and inflamed gingiva suggested an additional approach to the subject of gingival repair. A correlated study of oxygen consumption and histological changes in the course of gingival healing was undertaken, therefore, in an effort to augment existing knowledge by revealing an additional aspect of the gingival repair process.

EXPERIMENTAL METHOD

The experimental procedure consisted of the following (a) an initial gingivectomy with determination of oxygen consumption and microscopic study of the excised tissue, and (b) removal of tissue from each area at different intervals in the course of healing with determination of oxygen consumption and microscopic changes. With this procedure it was possible to correlate oxygen consumption and microscopic changes in the course of healing following gingivectomy.

Seventeen dogs, 10 males and 7 females, ranging in weight from 16 to 22 pounds and maintained on a diet of Gaines Dog Food and water, were used as experimental animals. The initial gingivectomies were performed in 50 areas. The elapsed interval between the first and second operation varied in different areas as follows: 5, 7, 9, 12, 14, 21, 39, 46, and 64 days. With the exception of 2 dogs, the tissue was studied after a single postoperative interval. In 2 animals the tissue was examined at two different postoperative intervals. Five days were used as the earliest postoperative interval because the friability of the tissue obtained earlier precluded valid oxygen consumption determinations.

The operations were performed under general anesthesia induced with veterinary Nembutal Sodium (Abbott) injected intraperitoneally, 1 c.c. per 5 pounds of body weight. The section of gingiva removed extended from the canine to the first molar and from the gingival margin approximately $\frac{3}{8}$ inch toward the mucobuccal fold (Fig. 1). After removal the gingiva was sliced with a razor blade so that the intended thickness of each slice was 0.5 mm. A portion of each specimen was also set aside for microscopic study. The sliced

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gingiva was placed in flasks containing 3 c.c. of phosphate buffered Ringer's solution (M.P.B.R.) pH 7.5. The flasks were equilibrated with oxygen at 37.5° C. for 15 minutes, and final readings were made after one hour at constant temperature. Each QO_2 determination represented the average of no less than 3 readings, the great majority of determinations being the averages of more than 5 readings; in all, 224 readings were made. The average interval of time between the excision of the tissue and the beginning of equilibration was 128 minutes, ranging from 45 to 331 minutes. The average dry weight per flask was 12.6 mg., ranging from 3.9 to 28.4 mg.

FINDINGS

I. Normal Gingiva

Normality was established by clinical and microscopic appearance. Microscopically the gingiva consisted of stratified squamous epithelium with pronounced rete pegs and slight surface keratinization and an underlying densely collagenous connective tissue stroma (Fig. 2).

Based on a total of 146 readings, the oxygen consumption for normal gingiva was established as 1.3, with a standard deviation of ± 0.07 (Table I). This is comparable to that of normal human gingiva (1.6 ± 0.37).[8] Slight variations in normal QO_2 were associated with microscopically detectable inflammation. The variations associated with inflammation were consistent with the findings in our previous study which indicated a comparable effect of inflammation upon oxygen consumption of human gingiva.

TABLE I

QO_2 VALUES OF NORMAL GINGIVA

DOG NO.	NO. READINGS	QO_2
1	8	1.5
2	9	1.1
3	6	1.2
4	9	1.0
5	12	1.6
6	10	1.3
7	8	0.9
8	11	1.5
9	12	1.6
10	8	1.5
11	5	1.4
12	6	0.8
13	11	1.1
14	6	1.2
15	8	1.8
16	7	1.7
17	10	1.4
	Average QO_2	1.3 ± 0.07

± = Standard deviation.

II. Healing Gingiva

Comparison of oxygen consumption values for the gingiva at the outset of the experiment with those at various stages in healing is shown in Table II. Oxygen consumption increased in the course of healing to reach a maximum of 5.4 on the fourteenth day. It then declined until at the twenty-first day it

TABLE II

OXYGEN CONSUMPTION OF HEALING GINGIVA

	PRE-OPERATIVE O_2 CONSUMPTION		O_2 CONSUMPTION IN THE COURSE OF HEALING		
DOG NO.	NO. OF READINGS	QO_2	DAYS OF HEALING	NO. OF READINGS	QO_2
1	10	1.4	5	4	2.8
2	7	1.7		7	3.5
3	6	1.3	7	3	4.0
4	8	1.8		3	2.7
5	8	1.5	9	8	3.1
6	10	1.4		5	2.8
7	12	1.6	12	8	4.5
8	5	1.4		4	3.3
3	5	1 0	14	4	5.4
4	4	1.9	21	4	2.1
11	6	1.2	39	9	1.8
12	8	1.5	46	9	1 5
13	9	1.1	64	10	1.9

approximated the preoperation level. With minor variations, the QO_2 value remained at the preoperation level from the twenty-first day through the remainder of the sixty-four-day experimental period.

Fig. 1.—Area of operation extending from canine to molar.

III. Correlation of Oxygen Consumption With Microscopic Changes in Course of Healing

The oxygen consumption of the gingiva in the course of healing following gingivectomy varied according to a pattern related to the histological changes. (An initial fall in oxygen consumption immediately after operation has been described in the skin.[3] The gingival specimens obtained earlier than the fifth day in the present experiment were unsuitable for satisfactory QO_2 determination.) The elevated QO_2 at the fifth day of healing was associated with an influx of inflammatory cells, proliferation of epithelium, and the forma-

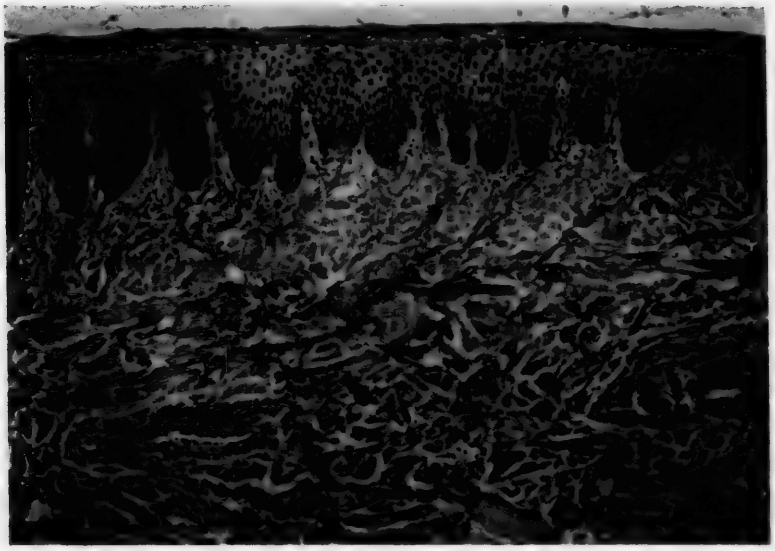

Fig. 2.—Normal gingiva showing slight surface keratinization of epithelium and underlying densely collagenous connective tissue stroma. (Hematoxylin and eosin, orig. mag. ×150.)

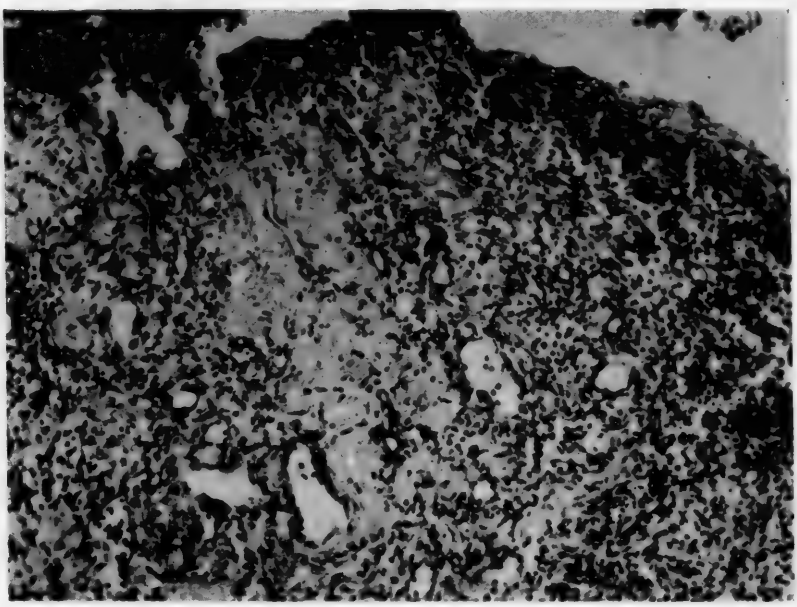

Fig. 3.—Fifth day of healing showing edema and diffuse leukocytic infiltration of connective tissue with new capillaries and filamentous collagenous fibrils. Newly formed surface epithelium presents slight edema and leukocytic infiltration. (Hematoxylin and eosin, orig. mag. ×200.)

tion of new capillaries (Fig. 3). The edema and degeneration present at this stage tend to depress the over-all oxygen consumption. An additional rise in QO_2 from the fifth to fourteenth day resulted from continued proliferation of epithelium, new capillary formation, fibroblastic activity, and a reduction in inflammatory and degenerative changes (Figs. 4 and 5). The reduction in QO_2 from the fourteenth to twenty-first day when the preoperation level was reached was associated with a reduction in vascularity and cellularity of the connective tissue, absence of fibroblastic activity, resolution of inflammation, and maturation of the epithelium. The preoperation QO_2 level attained at the twenty-first day was maintained with slight variation throughout the remainder of the sixty-four-day experimental period, in the course of which the microscopic appearance of the gingiva was essentially that observed before operation.

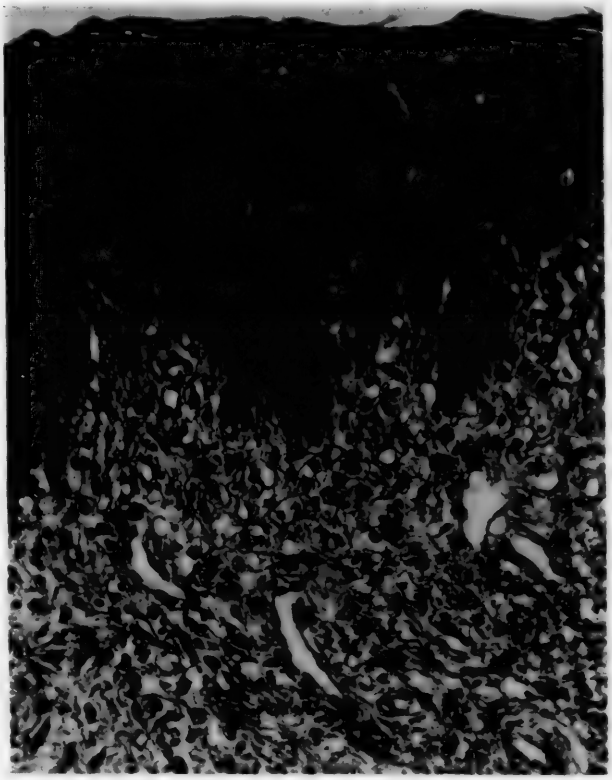

Fig. 4.—Ninth day of healing showing well-formed epithelium. The connective tissue presents numerous fibroblasts and capillaries, collagen fibrils, scattered leukocytes, and edema. (Hemotoxylin and eosin, orig. mag. ×240.)

DISCUSSION

The findings suggest that it is the nature of the tissue changes in the reparative process that determines the oxygen consumption and that it is not the

oxygen supply which governs the sequence of cellular changes. In the technic employed for the determination of oxygen consumption in this study the gingiva is bathed in a medium saturated with oxygen so that the pressure gradient of oxygen between the medium and the tissue is at a maximum during the period when the tissue is metabolizing. Nevertheless, the tissues obtained at different stages of repair use different amounts of oxygen according to the metabolic requirements of the cellular components. The relationship of oxygen consumption to cellular activity exhibited by healing gingiva is in accord with the concept of Best and Taylor,[9] who note that whereas increased activity of a tissue entails an increase in oxygen consumption, increase in the oxygen supply above the tissue requirements does not increase oxygen usage. The present findings discourage the assumption that the artificial introduction of oxygen would hasten appreciably the sequence of cellular changes involved in gingival healing.

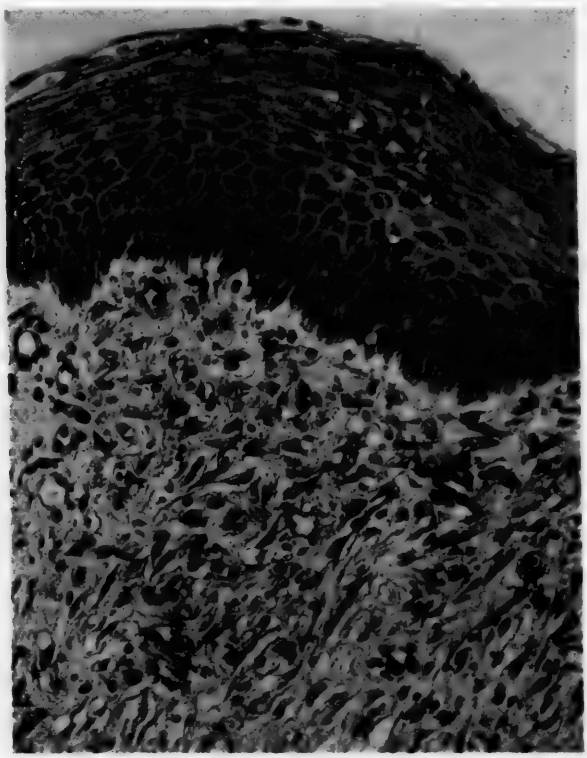

Fig. 5.—Fourteenth day of healing showing prominent distribution of collagen, presence of mature fibrocytes and fibroblasts, slight edema, and very few leukocytes. (Hematoxylin and eosin, orig. mag. ×240.)

Normally, a greater demand of tissues for oxygen is met by increasing the total blood flow through the tissue, thus maintaining a high intracapillary oxygen pressure, and by raising the coefficient of oxygen utilization, that is, in-

creasing the quantity of oxygen abstracted from a given volume of blood.[9] A steep oxygen pressure gradient between plasma within the capillaries and the tissue cells increases the coefficient of oxygen utilization. Nature increases the pressure gradient by increasing the metabolic end products such as carbonic and lactic acids, by lowering the oxygen tension within the tissue cells, by causing a rise in the temperature of the tissue, and by shortening the distance through which the oxygen must diffuse, that is, by opening up more capillaries and thus reducing the radius of tissue supplied by each capillary. Our findings reveal no microscopic evidence of circulatory inadequacy which might be construed as impairing the healing processes by interfering with the oxygen requirements of the tissues.

CONCLUSIONS

A correlated study of oxygen consumption and microscopic changes in the healing gingiva of dogs following gingivectomy revealed the following:

1. The QO_2 of the normal gingiva of the dog (1.3 ± 0.07) is comparable to that of the human being (1.6 ± 0.37).

2. Oxygen consumption in different stages of healing following gingivectomy varies in accordance with the nature of the microscopic tissue changes.

3. Oxygen consumption is elevated in the course of gingival healing until the maximum QO_2 is reached at the fourteenth postoperative day. From the fourteenth to the twenty-first day, there is a decline in QO_2 to approximately the level of normal gingiva.

4. The findings discourage the assumption that the artificial introduction of oxygen would hasten appreciably the normal cellular processes in the course of gingival healing.

The authors are indebted to Dr. David Rapport and Dr. Atillio Canzanelli of the Department of Physiology of Tufts College Medical School without whose assistance this study could not have been conducted.

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BACTERIAL GROWTH AND CRYSTAL FORMATION: A POSSIBLE FACTOR IN CALCULUS FORMATION

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A PHENOMENON of bacterial metabolism is the formation of crystals in certain types of media. Recently, while the colonial characteristics of several strains of oral actinomycetes isolated from dental calculi were being studied, by means of low magnification, crystals of ammonium magnesium phosphate were observed in the immediate vicinity of the colonies. The crystals were either within the colonies, beneath them, or neighboring their peripheries. No crystals were observed in those portions of the medium in which no bacterial growth occurred. The medium employed was a beef heart infusion agar with 10 per cent horse blood. Inoculation of the oral actinomycetes was by streaking the surface of the medium, and the medium was incubated at 37° C. aerobically for five days.

Several references to crystal formation were found in the bacteriological literature. Scudder (1926) reported the presence of ammonium magnesium phosphate crystals in a semisolid beef heart infusion medium. The crystals were found in the zone of growth of various bacterial species. Scudder concluded that crystal formation was a result of bacterial activity in an alkaline medium and that it varies according to the reaction, water of condensation, and species of bacteria. Huddleston (1927) also observed NH_4MgPO_4 crystals in cultures of Brucella grown on liver infusion semisolid medium. The crystals appeared after four to five days' incubation and were visible along the line of growth and later beneath it. The crystals formed at pH 7.2 to 7.4 but not in an acid environment. If the inoculated medium was kept in a jar under 10 per cent carbon dioxide tension, good growth of the microorganism occurred but no crystals developed. However, when such cultures were removed from the jar and the carbon dioxide volatilized, then the culture became alkaline and crystals formed in the neighborhood of the colonies. Huddleston claimed that the bacterial enzymes decompose the amino acids in the medium liberating ammonia thereby rendering the medium alkaline. The magnesium and phosphate ions are components of the liver infusion and these combine with the ammonia to form ammonium magnesium phosphate crystals. Buchanan and Fulmer mentioned the occurrence of ammonium magnesium phosphate crystals in bacterial cultures as well as ferric iron compounds and carbonates and oxalates of calcium. Further study of this phenomenon of crystal formation was considered warranted in that (1) this was the first noted observation of crystal formation by oral actinomycetes and (2) may add further corroboration to Naeslund's (1926) theory of the activity of actinomycetes in the formation of dental calculi.

Experiment I.—To determine whether variation in the pH of the medium would affect the deposition of crystals, a beef heart infusion agar was prepared

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(beef heart infusion, 1000 ml., Difco peptone, 10 Gm., NaCl 5 Gm., agar 20 Gm., and adjusted to pH 7.0. 30 ml. of phenol red indicator was added). Horse blood was not used since the actinomycetes after isolation grew just as well without it. The medium was divided into two portions and to one of these dextrose was added in an amount to give a concentration of 1 per cent. After sterilization plates were poured from each portion. The oral actinomycetes were streaked upon the surface of the respective portions and the media incubated at 37° C. aerobically for 5 days.

Crystals of ammonium magnesium phosphate were found only on the medium containing no dextrose. The phenol red indicator in the vicinity of the colonies was a slight pink indicating a reaction approximating pH 7.2 to 7.4. The medium to which the dextrose was added was yellowish in color indicating a reaction of pH 6.8, or lower, due to the fermentation of this sugar by the microorganisms. No crystals were evident at this reaction.

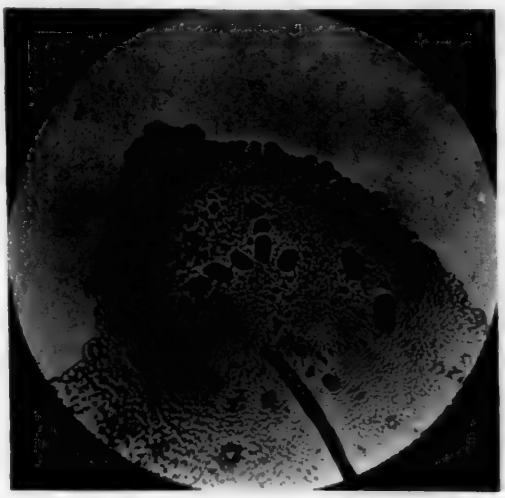

Fig. 1.—Ammonium magnesium phosphate crystals in growth zone of an oral actinomycete. (Orig. mag. ×40.)

Experiment II.—To determine whether change of surface tension due to streaking the surface of the medium would induce crystal formation, one-half of the beef infusion agar contained in plates was streaked with the oral actinomycetes. The other half of the medium was streaked in similar fashion with a sterile loop. After five days' incubation, examination disclosed the presence of crystals about the actinomycete colonies but none in the vicinity of the sterile streak lines. An indication that crystal formation is dependent on microbic metabolism.

Experiment III.—To determine whether drying of the medium affected crystal formation, plates of beef heart infusion agar, inoculated and uninoculated, were kept at room temperature for two weeks after one week incubation at 37° C. Crystals were only found on the inoculated plates in the vicinity of the colonies.

Experiment IV.—To determine whether crystal formation occurs in the presence of a mixed bacterial flora, the saliva of twelve individuals was collected in sterile jars and immediately streaked across the surface of beef heart infusion heart plates containing phenol red indicator. After one week's aerobic incubation crystals were observed about the streak lines of growth on the various plates. The phenol red indicator in the area of growth was slightly pink indicating a pH approximating 7.2 to 7.4. This result would seem to indicate that crystal formation may occur in a mixed bacterial population provided the reaction is alkaline as a result of decomposition of proteins contained in the medium.

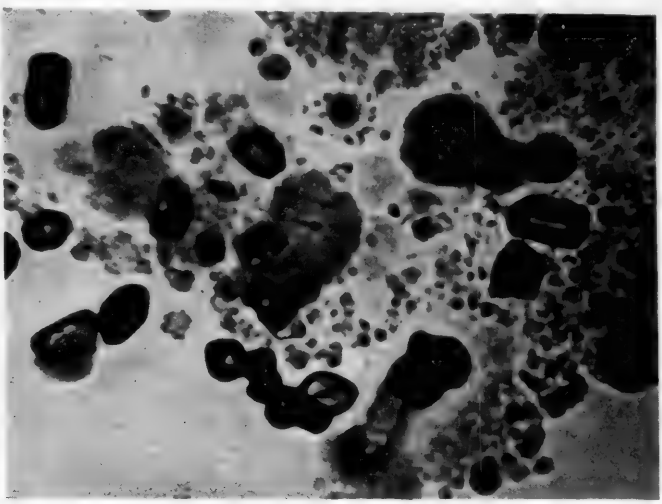

Fig. 2.—Ammonium magnesium phosphate crystals and actinomycete colonies. (Orig. mag. ×40.)

DISCUSSION

The production of salivary calculus according to Naeslund is dependent on the metabolic activity of actinomycetes. These microorganisms decompose proteins by their enzymes with a local change of reaction to alkaline which induces the deposition of calcium salts from the saliva. Another characteristic of the actinomycetes is their ability to adhere to smooth surfaces. Indeed, broth cultures may show colonies clinging to the walls of the test tube. Rosebury and Karshan (1938) state . . . "Of these theories advanced to explain formation of salivary calculus, the one that appeared most consonant with the data is that of Naeslund." Tennebaum and Karshan (1944) have evaluated some of the chemical constituents of the saliva of calculus-forming and non-calculus-forming individuals. They found that the calculus-forming group had higher concentrations of calcium and phosphates as well as a slightly higher pH (alkalinity). A local increase in the alkalinity of the saliva as suggested by Naeslund's studies would, according to these investigators, result in the precipitation of insoluble calcium phosphate.

Since the inorganic components of calculus are primarily calcium phosphate and calcium carbonate with smaller amounts of the salts of magnesium

and other elements (Tennebaum and Karshan), the formation of ammonium magnesium phosphate in the oral cavity would necessarily be limited. However, the process by which deposition of the inorganic salts of calculus or the formation of ammonium magnesium phosphate crystals occurs, namely, an increase in local alkalinity resulting from the decomposition of proteins by the enzymes of the actinomycetes, is apparently identical. Therefore, the in vitro formation of crystals is of interest as a supplemental fact in the corroboration of the Naeslund theory of calculus formation.

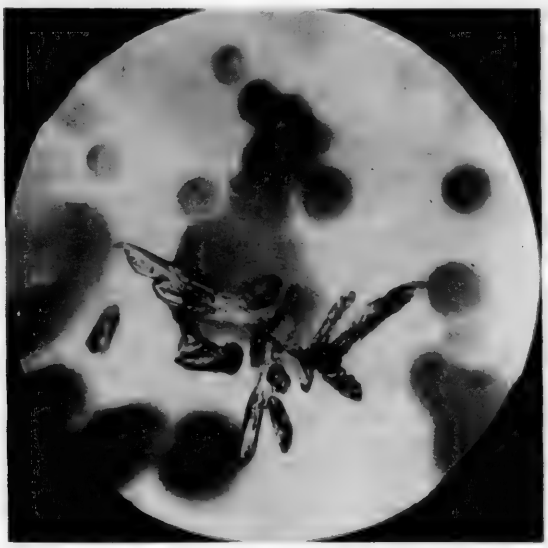

Fig. 3.—Group of crystals which initially started from nidus of one. (Orig. mag. ×20.)

SUMMARY

The production of ammonium magnesium phosphate crystals about colonies of oral actinomycetes has been observed. Crystal formation resulted from an increase in alkalinity caused by decomposition of protein with liberation of ammonia which combined with the magnesium and phosphate components of the medium. This phenomenon is mentioned as a further corroboration of Naeslund's theory of salivary calculus formation.

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PHYSIOLOGICAL TOOTH MIGRATION AND ITS SIGNIFICANCE FOR THE DEVELOPMENT OF OCCLUSION

IV. The Biogenesis of Overbite

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IN THE field of periodontics a great deal of interest has been directed toward the problem of the incisal overbite. Frequently a severe overbite has been held responsible for progressive periodontal lesions or marked abrasions which emphasizes the need for early prevention of this malocclusion. In the search for the causative factors of this condition conflicting schools of thought exist. There are those who believe that it is due to a lack of growth in vertical height of the mandibular ramus.[10] On the other hand, Strang[18] suggests that its cause is a supraeruption of the incisors or an infraeruption of the posterior teeth, or a combination of both. Infraeruption of the permanent molars alone has been proposed by others.[13, 14] In two cephalometric studies of the relationship between ramus height, dental height, and overbite, Wylie[21, 22] came to the conclusion that growth of the mandibular ramus has no direct relationship to the development of the overbite. This was consistent with the observations of Broadbent[7] and Brodie[8] that the form and proportion of the mandible during development remain constant. It further sustains the concept of Thompson and Brodie[20] that the presence of teeth has little bearing on the rest position of the mandible.

An effect of the incisal overbite on the form of the temporomandibular joint was found in morphological as well as histological examinations.[6, 12, 17] This problem has recently been studied by Angel.[1] Baker[2] suggested that the eruption sequence of the canines and premolars influences the formation of the permanent overbite. With the exception of Goldstein and Stanton,[11] Sillman,[15] and Silver[16] little information, however, is presented on the development of overbite in the same individual.

The following study deals with the relationship between the degree of overbite of the deciduous dentition and that of the mixed and permanent dentitions and the factors responsible for a transitory change, if any, from the deciduous overbite to the permanent overbite in the same individual.

MATERIAL AND METHODS

The material on which this study is based consists of 52 series of plaster reproductions as described in the previous papers.[3, 4, 5] Casts of each case were made before and after eruption of the permanent incisors and were classified according to the degree of overbite as checked from the lingual aspect of the plaster reproductions in occlusion. The overbite was designated as "slight," if the incisal edges of the mandibular first incisors met the upper incisal third of the crowns of the opposing maxillary incisors. If the lower first incisors occluded with the middle third of the upper incisor crowns the overbite was called

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"medium." It was recorded as "severe" when the mandibular incisors met the protuberance of the cingulum or were found to strike the gingiva.

Measurements were made of the length of the frontal portion of the upper and lower arches in the deciduous and the mixed dentures, and the amount of forward extension of the maxillary and mandibular arches was calculated from these data.

RESULTS

Table I shows the distribution of the different degrees of overbite in the deciduous and the following mixed dentition. In the deciduous dentition 21 cases (40 per cent) showed a slight overbite, 15 cases (29 per cent) a medium overbite, and 16 cases (31 per cent) a severe overbite.

TABLE I

DISTRIBUTION OF THE DEGREES OF OVERBITE OF 52 CASES IN THEIR DECIDUOUS AND MIXED DENTITION

	DECIDUOUS DENTITION		MIXED DENTITION	
OVERBITE	NO.	%	NO.	%
Slight	21	40	10	19
Medium	15	29	14	27
Severe	16	31	28	54

As shown previously[3] the overbite in the deciduous dentition remained constant except when subjected to environmental influences such as attrition and habits. According to Table I the degree of overbite changed materially as the cases progressed from the deciduous dentition to the mixed dentition. Slight overbite was found in 10 cases; its percentage decreased from 40 per cent in the deciduous dentition to 19 per cent in the mixed dentitions. The number of cases with a medium overbite remained about the same while those with a severe overbite increased from 16 to 28 cases (54 per cent).

TABLE II

RELATIONSHIP OF OVERBITE BETWEEN THE DECIDUOUS AND PERMANENT DENTITION IN 52 CASES

DECIDUOUS TO MIXED DENTITION	NUMBER	PER CENT
Less overbite	5	10
Same overbite	22	43
More overbite	25	47

Table II shows the relationship of overbite between the deciduous and mixed dentition in 52 cases. Only 5 cases (10 per cent) showed in the mixed dentition less overbite than in the deciduous dentition. No case with *severe* deciduous overbite was seen to develop into a *slight* permanent one. Twenty-five cases (47 per cent) retained the same incisal relationship, while 22 cases (43 per cent) developed more overbite in the mixed dentition. Some cases which had an end-to-end relationship in the deciduous denture showed a severe incisal overlapping in the mixed dentition. The data of Table I and II reveal that *during the transition of the deciduous into the mixed dentition there is a developmental tendency toward the formation of a deeper overbite.*

Since there was lessened forward extention of the mandibular arches during the same developmental period,[5] the possibility of a correlation between forward

growth and overbite was checked. The measurements of the forward extension of the upper and the lower arches were compared in each case with the development of its overbite. Thus a biogenetic mechanism which determined the degree of overbite became apparent. The findings may be stated as follows:

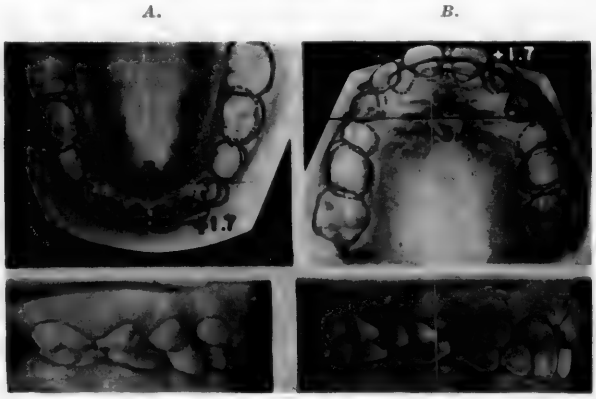

Fig. 1.—The deciduous and mixed arches of Case 27 are superimposed on a line connecting the distals of the deciduous canines.

A, The mandibular forward extension is 1.7 mm.

B, The same forward extension of 1.7 mm. occurred in the maxillary arch.

C, Note slight overbite of the deciduous dentition.

D, Slight overbite is also seen in the mixed dentition.

Fig. 2.—Slight overbite in the deciduous dentition is followed by a severe overbite in the mixed dentition concomitant with insufficient forward extension of the mandibular arch is seen from the lingual aspect of the plaster reproduction of 3 cases:

		A, Case 4	*B*, Case 9	*C*, Case 38
Forward extension	Upper arch	3.0 mm.	3.5 mm.	4.0 mm.
	Lower arch	0 mm.	1.0 mm.	1.0 mm.

1. If the mandibular forward growth was approximately the same (± 0.5 mm.) as the maxillary forward growth, the same incisal overbite was produced in the mixed dentition. Fig. 1 represents an example of such a development where the slight overbite in the deciduous dentition was followed by a slight overbite in the mixed one. The mandibular forward extension in both arches was 1.7 mm.

2. If there was no forward extension of the mandibular arch or less than that of the maxillary arch, a deeper incisal overbite was observed. Fig. 2, *A* to

C show such instances where the slight overbite in the deciduous dentition due to the lack of mandibular forward growth had developed into a severe overbite in the mixed dentition.

3. If the forward extension was greater in the mandibular than in the maxillary arch, less overbite was produced in the mixed dentition than in the deciduous one. An analysis of the models of Case 25 in Fig. 3, *A* to *E* demonstrates the key role of mandibular forward growth in the development of the overbite. The medium overbite of the deciduous denture was followed by a severe overbite in the mixed denture. The mandibular forward extension was only 0.5 mm. whereas the maxillary one was 3.0 mm. During the eruption of the lower second premolars and upper permanent canines a marked decrease of the incisal overlapping occurred. The cause of this change is indicated by measurements which show a forward development of the mandibular arch by 2.5 mm. and none of the upper arch. This is also apparent with the interdigitation of the first premolars in Fig. 3, *D* and *E*. The upper arch developed more transversally through a widening of the intermolar distance by 3.0 mm.

Fig. 3.—Development of overbite in Case 25.

A, Note medium overbite in the deciduous dentition.

B and *C*, Note severe overbite in the mixed dentition. Due to premature loss of deciduous molars, the anterior portion of the lower arch moved only 0.5 mm. forward, while the upper arch developed 3.0 mm. forward.

D and *E*, Note marked decrease in overbite upon eruption of the lower second premolars and compare development of intercuspidation of the opposing first premolars.

The study of this series reveals a definite effect of the eruption sequence of the canines and premolars on the degree of the permanent overbite. It was observed that in the mandible the permanent canines erupted almost consistently before the second deciduous molars were shed. The lower permanent canine which is about 1.5 mm. wider than its predecessor, therefore, had to secure additional space through a forward extension of the anterior portion of the arch. The extra space, caused by the greater width of the deciduous molars in comparison with that of their successors later was filled through the secondary

mesial migration of the permanent molars, which already has been described as the "late molar adjustment."[4]

In the maxillary arch the first premolar ordinarily erupts before the canine and second premolar. The result of this eruption sequence is that room for the wider permanent canine can be provided from the space reserve of the shed deciduous molars; the already erupted upper first premolar then is pushed distally. Hence, in the maxilla there was less forward growth than in the mandible; this mechanism aided in decreasing the overbite. Fig. 4 illustrates schematically the order of the physiological tooth migration and its effect on the occlusion. According to both types of deciduous arch forms two different modes of further development are shown. Common for both, however, is the relative constancy of the deciduous canine axis.

DISCUSSION

The degree of overbite in the deciduous dentition appears to be one of the determining factors in the formation of the permanent overbite. Severe overbite in the deciduous denture had a definitely unfavorable prognosis while slight overbite generally was followed by a somewhat increased one in the mixed dentition. The degree of overbite, however, seems to depend primarily upon the amount of mandibular forward growth. This takes place at three different periods: (a) During the eruption of the deciduous canines when it apparently determines the overbite of the deciduous denture; (b) during the eruption of the permanent incisors at which time it may regulate the overbite of the mixed denture; and (c) during the eruption of the permanent canines and premolars when it may define the degree of the permanent overbite.

The downward growth of the upper incisors is guided by the incisal edges of the lower opponents due to the time interval between the eruption of the upper and the lower teeth. The more the lower incisors remain lingual to the uppers, the further the uppers move downward thereby increasing the overbite. This form of development does not allow for enough clearance in the posterior region to facilitate sufficient vertical growth of the alveolar processes during eruption of the accessional teeth.* Serial observations therefore suggest the revision of the contention that incisal overbite should depend upon the amount of eruption of the permanent molars.[13, 14, 18] Therapeutical experience teaches also that a deep overbite cannot be decreased permanently by a proper vertical opening of the bite without a sagittal component. Physiologically this consists in the forward growth of the mandibular alveolar process and not in a mesial displacement of the mandible.

A parallel phenomenon to the tendency toward an increased overbite in the ontogenetic development is well known in the phylogenetic history. Among prehistoric and historic skulls of men of northern Europe incisal overlapping is seldom found. Its incidence increased progressively with the beginning of the fifteenth century and became the orderly form of modern man. A significant reduction in mandibular arch length became concomitant.[10]

*The downward growth of the roots into the corpus mandibulae of some lower already occluding first permanent molars in the report of Carlson[3] may find an explanation in a deep incisal overbite.

The present data may serve as a warning against the recent trend in the field of orthodontics to remove permanent teeth in the mandibular arch, without considering its effect on the incisal overbite. It does not agree with the attempt to justify extractions by comparative measurements of the widths of the

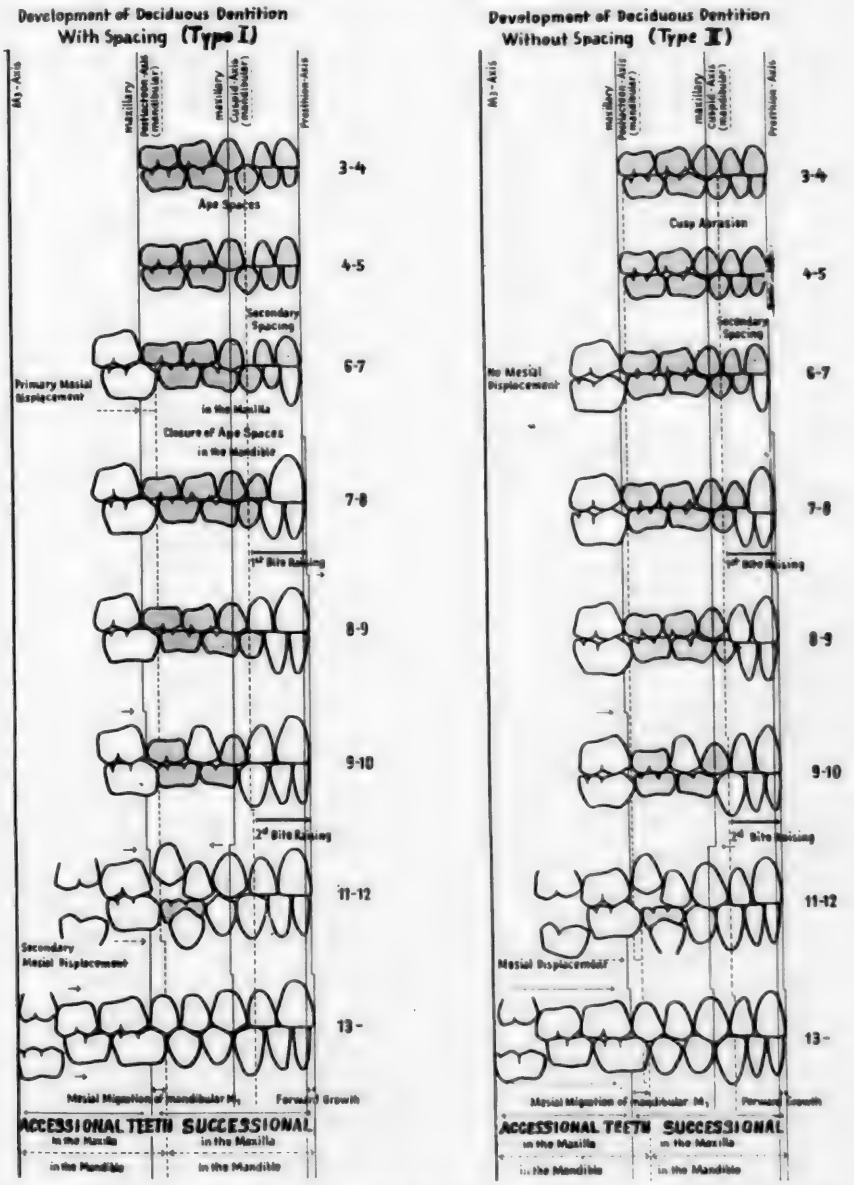

Fig. 4.—Illustration of the physiological tooth migration in the sagittal plane, the result of a biometrical survey on 60 series of development.

deciduous molars and their unerupted successors. That procedure doesn't take into account the possibility of a physiological forward growth concomitant with the eruption of successional teeth.

Models alone naturally are not sufficient to demonstrate the role of growth of the mandible upon the development of overbite. These and the other data,[3, 5] however, seem to confirm the concept that the alignment of the teeth and the development of the alveolar processes follow along lines not identical with the growth rate of the other parts of the jaws. When, during the transition into the mixed dentition, the overbite became more or less pronounced or even a disto- or mesioclusion was produced, no distal or mesial shift of the mandible could be observed. The mesiodistal relationship of the opposing deciduous canines remained constant. This is in keeping with the reports[21, 22] that the development of an overbite has no direct relationship with the growth center of the mandible.

SUMMARY

In a survey of 52 cases the development of overbite was studied. The following summarizes the findings.

1. The degree of incisal overbite in the permanent dentition primarily was determined by the extent of mandibular forward growth which occurred during the eruption of the successional teeth. The lessened forward extent of the mandibular arch was responsible for a greater incidence of severe overbite in the mixed dentures.

2. The deciduous overbite was also a determining factor in that the slight overbite tended to increase during the period of the mixed dentition while a severe overbite became worse.

3. The definite overbite of the permanent dentition finally depended upon the eruption sequence of the permanent canines and premolars. If in the lower arch the sequence was canine, first premolar, second premolar and in the upper arch first premolar, canine and second premolar, the best results were observed.

4. Incisal overbite developed independently of the mechanism of molar adjustment.

GENERAL CONCLUSIONS

(Including Papers I to III of this series)

A biometrical study of the development of the dental arches and occlusion in 60 children revealed the occurrence of several physiological mechanisms which influenced the final position of the teeth.

1. Considerable migration of the teeth *concomitant* with the eruption of accessional and successional teeth was observed to effect the increase in dimensions of the arches. Teeth and alveolar process thus are a genetic entity.

2. Observations made in this study endorse Brodie's principal concept that the development of the dentition is mainly based upon a congenital pattern. This pattern predetermines the spaced or closed arrangement of the deciduous arches and the positioning of the permanent tooth germs which seem to be of greater significance than function.

3. Differentiation between the manifestation of the general growth of the facial skeleton and that of the alveolar processes is conducive to a better understanding of the etiology of malocclusion and the development of certain paradentopathies.

4. Successful methods for preventive treatment may be instituted as a result of a thorough knowledge of the physiological migration of the teeth.

The author wishes to express his appreciation to Dr. H. Becks for his support and encouragement, and to Drs. J. Haber and H. Ray for assistance in preparation of this series of papers.

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MASTICATORY PERFORMANCE AND EFFICIENCY

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SIMPLE, reliable methods for measuring masticatory function would be useful aids in evaluating the success of dental restorative procedures. One goal of dental restoration is to improve the masticatory function of patients who have lost teeth. It is surprising how little is known by the dentist concerning his achievement of this goal. Current practices are based on the satisfaction of the patient or the fulfillment of certain theoretical and arbitrary standards that have become accepted because of the clinical experience of their proponents. Tooth structure is replaced wherever it has been destroyed; occlusal surfaces are carved to provide considerable contact with opposing teeth and to approximate ideal tooth form. The completed restorations are never checked objectively for their masticatory function and there are not even data to prove that the ideal tooth form will provide best function under all conditions. For example, flatter cusps might be advantageous in masticating processed foods, and the occlusal shape might be modified to compensate for missing teeth. Perhaps contact area should be diminished on teeth which cannot tolerate forces readily, and the normal occlusal pattern might be discarded entirely for denture teeth among cases where forces are severely limited.

Several investigations of this topic have appeared in the foreign literature but only a few in English, and there is no agreement concerning the test to be used. The procedures are complicated and vary in the foods and technics that are recommended. Investigators have been unable to agree as to which of several criteria should be used and have been puzzled by the wide variation in performance among persons having the same numbers and types of missing teeth.

Most authors agree concerning the principles to be employed in designing a function test for mastication. The test should be selected from foods that are normally consumed. These foods should offer the proper degree of difficulty, permitting a normal dentition to receive a high rating and a deficient dentition, a poor rating. Any selective action that is performed by the normal and is missing from the deficient mouth should be taken into account. A certain degree of precision should be obtained, and the method should be simple, rapid, and inexpensive.

Masticatory function tests have been described by Lehman,[1] Gaudenz,[2] Christiansen,[3] Schutz,[4] Paulsen,[5] Claussen,[6] Balters,[7] Ascher,[8] Gelman,[9] Juul,[10] Ono,[11] Sognnaes,[12] and Dahlberg.[13] A variety of test foods were proposed, including Spanish hazel nuts, Brazil nuts, sweet almonds, boiled egg white, potato, apple, carrot, bread, zwiebach, turnip, coconut, meat, cracked corn, and

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hardened gelatin. Except for Ono's studies on meat, the masticated food was usually sifted through one or more sieves. It was generally observed that dentitions judged to be clinically adequate caused a finer pulverization of the food than those judged to be inadequate, but the relative difference between adequate and inadequate dentition was found to depend upon the test food employed and screen size which was selected as the best criterion of masticatory performance. There was no consensus on these questions.

The purposes of the investigation were to study the particle size distribution of test foods in an effort to learn what criterion should be used as an indication of masticatory performance, and to evaluate the effect of loss of teeth on performance.

EXPERIMENTAL PROCEDURE

For preliminary tests, four foods were selected which seemed to be suitable from the standpoint of low cost and uniformity in size and hardness. These were salted peanuts, shredded coconut, carrots, and raisins. Their suitability for sieve analysis was tested. It was found that 80 per cent of the weight of the peanuts could be recovered when five 3-gram portions were subjected to 20 masticatory strokes on each portion, sieved, collected on filter paper, and dried. Only 20 per cent of the original weight was lost by swallowing, by solution or emulsification of food to permit its passage through the filters, or by loss in the moisture content of the food. Loss with shredded coconut amounted to 40 per cent. The moisture content of carrots and raisins was so large that over 90 per cent of the original weight was lost, and it was concluded that these foods were not suitable for study by gravimetric sieve analysis.

The following procedure was employed for determining the particle size distribution of masticated food. A 15-gram sample of peanuts* was divided into five 3-gram portions. The subject was instructed to masticate each portion a specified number of times on one side of his dentition and to avoid swallowing any of the food. The chewed food was spit out into a beaker containing 50 c.c. of 0.3 per cent detergent solution.† He was also instructed to rinse his mouth twice with water and to add the rinsings to the beaker. When the five portions had been masticated, the volume of the expelled material and rinsings usually amounted to 200 to 300 c.c. It was stirred gently with a glass rod to break up clumps and poured quickly onto a nest of sieves consisting of 10-, 20-, 40-, 80-, and 200-mesh U. S. Standard screens. Fine particles were washed through the 10-mesh sieve with a jet of water from a wash bottle, and the portion on this sieve was transferred by washing with water to a tarred filter paper in a funnel. Next the fraction on the 20-mesh screen was washed free of finer particles and transferred to a separate filter. The same procedure was repeated with the portions retained by the 40-mesh screen but not for the 80- and 200-mesh screens, since the last two screens were judged to be sufficiently rinsed. Approximately 500 c.c. of water were required for sieving and 60 c.c. for transfer from each

*Planters Jumbo Salted Peanuts, vacuum-packed in 8 oz. cans.
†Dreft, manufactured by the Procter and Gamble Company.

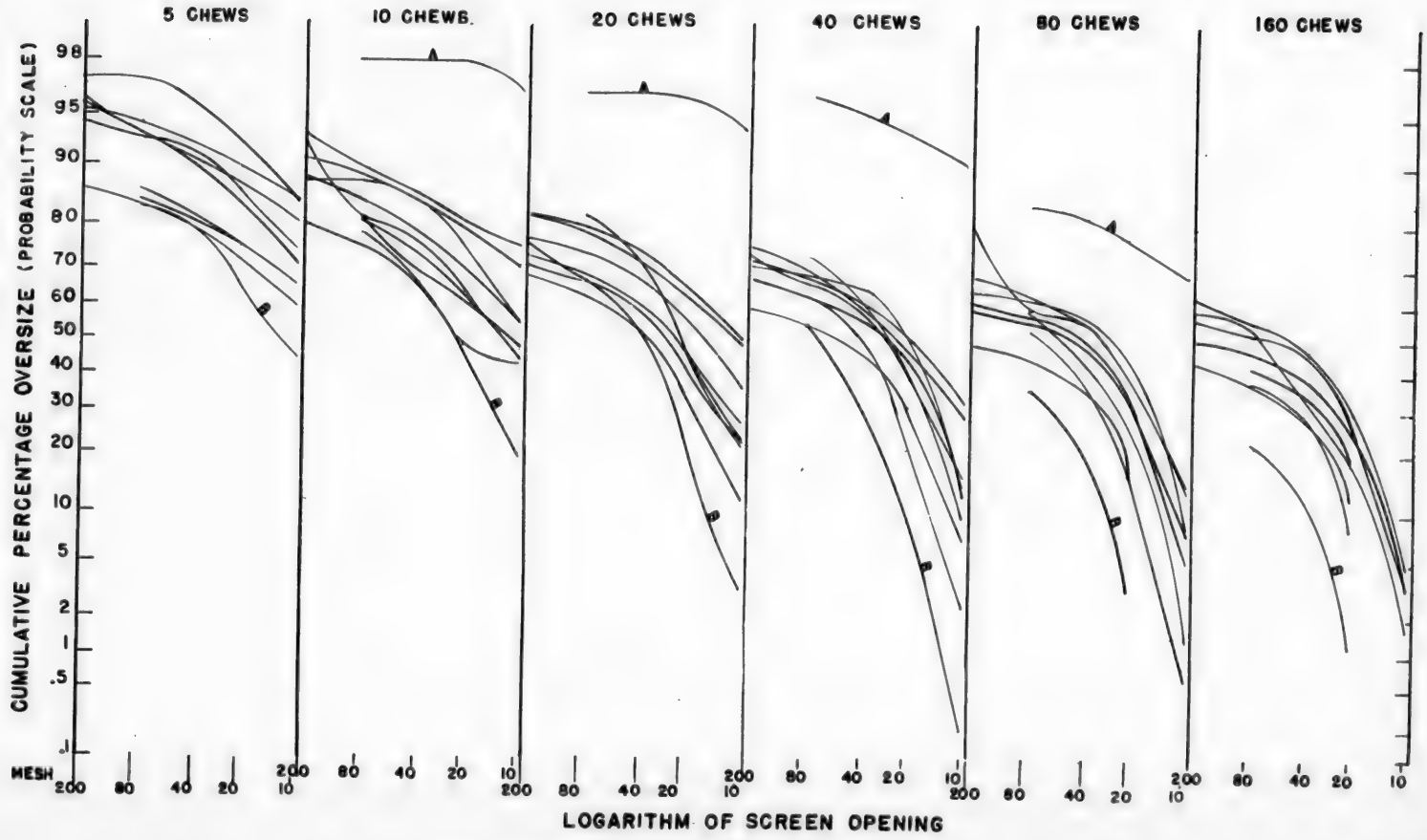

Fig. 1.—Particle size distribution of masticated peanuts.

sieve to the filter paper. The filters were dried in an oven at 100° C. for three hours, transferred to a desiccator for two hours, and weighed to the nearest centigram.

The total weight of peanuts recovered on all of the filters was divided into the weight found on each screen in order to obtain the percentage remaining on each screen. These percentages were added in the proper fashion to obtain the cumulative percentage oversize for each screen size.

Preliminary tests were made on a single individual at 20 and 40 chews per portion of peanuts. The results disclosed the fact that we were confronted with the same problem that had puzzled other investigators, a greater percentage of food passed through every one of the screens when the number of masticatory strokes was doubled, but the relative increase in pulverization was different for each screen. At this point there appeared to be no basis for selecting which screen size should be used as the criterion.

This quandary led to a study of the literature dealing with particle size distribution of ground minerals. This subject has been reviewed by Austin.[14] He demonstrated that particle size distribution is linear when the logarithm of the particle size is plotted against the cumulative percentage oversize on the probability scale. Hatch and Choate[15] demonstrated that this rule held for pulverized silica granite, calcite, and limestone. Austin disclosed that it was valid for crushed quartz, clay, soda ash, sodium bicarbonate, and alumina. His calculations were based on data obtained by others.[16-19] It seemed important to determine whether the particle size of masticated peanuts would also obey this rule.

Experiments were planned to disclose whether particle size distribution changes as mastication proceeds and whether it varies with different subjects. Subjects were selected whose half dentitions ranged from a single first premolar in occlusion to a complete dentition. Each subject masticated the 3-gram portion of peanuts in separate experiments for 5, 10, 20, 40, 80, and 160 masticatory strokes. The results are presented in Fig. 1 in the form of 57 distribution curves. On the ordinate is plotted the cumulative percentage oversize on the probability scale. On the abscissa is given the logarithm of the screen opening in millimeters. The size of screen was obtained from handbook data on the U. S. Standard sieves.[20]

The lines joining points for the experiment at 5 chews are nearly straight within the limits of variation for individual tests. The lines joining points at 10, 20, 40, 80, and 160 chews appeared to increase in curvature in the order mentioned. The curve for subjects *A* and *B* show abnormally low and abnormally high masticatory performance and could not be averaged with the remainder of the group for mathematical reasons.

The data for the remaining eight subjects were averaged and presented in Fig. 2. At 5 chews the average particle size distribution is almost a straight line. The average curves for 10, 20, 40, 80, and 160 strokes deviate progressively from a straight line but deviation takes place only in the 10- and 20-mesh portion of the curve. The 40-, 80-, and 200-mesh portions of the curves remain nearly straight and parallel. It may be concluded that the particles remaining on the

10- and 20-mesh screens disappear more rapidly than would be expected from a random grinding process. This indicates that mastication is a selective process which tends to produce subdivision of particles of peanuts greater than 0.84 mm. in diameter without a corresponding grinding of the finer particles. Because of this finding it was decided that performance tests with peanuts should be based on percentage of food passing the 10- or 20-mesh screen. The remainder of the screens could be eliminated. With the 10- or 20-mesh screen, the selective action of mastication to be evaluated and in addition the sensitivity of the tests would be greater than with finer screens. For example, the percentage oversize on the 10-mesh screen dropped 70 units between 5 and 160 chews. Whereas that on the 200-mesh screen decreased by only 39 units over the same range.

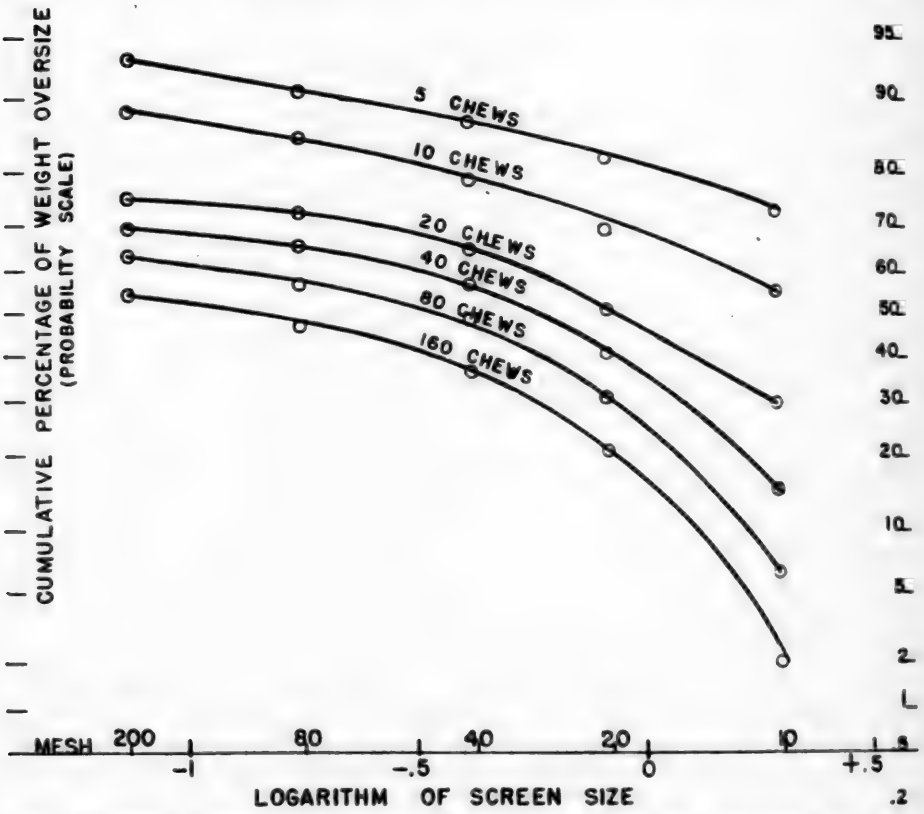

Fig. 2.—Average particle size distribution of masticated peanuts in performance of eight subjects.

The procedure adopted for masticatory performance was the same as that used in the particle size distribution test with two exceptions. Each subject masticated each portion of peanuts for 20 masticatory strokes and each test made use of the 10- and 20-mesh screens only. The masticatory performance (P) was

calculated in most instances as the percentage of food passing the 10-mesh screen. For some purposes the percentage passing the 20-mesh screen was calculated, but this has been specially designated.

RESULTS

Precision of Method.—Three tests were made on each of five subjects during the same day, after breakfast and before and after lunch. The standard deviation ranged from .6 to 2.4 masticatory units with an average of 1.6. Averages of tests made after breakfast and after lunch were in good agreement. The performance before lunch averaged 2.1 per cent higher than after meals which may indicate that hunger tends to increase a person's performance in mastication. Tests were made on different days in quadruplicate for three persons. Under these circumstances the standard deviation was 2.9 units, which suggests that variation from day to day is somewhat larger than diurnal variation.

TABLE I

MASTICATION CORRELATION COEFFICIENTS

TYPE OF FOOD USED	MESHES EMPLOYED	NUMBER OF SUBJECTS	NATURAL DENTITION OR DENTURE	CORRELATION COEFFICIENT	ONE PER CENT SIGNIFICANCE LEVEL
Peanuts	10-mesh + 10-mesh	25	natural	0.91	0.496
Peanuts	20-mesh + 20-mesh	25	natural	0.92	0.496
Peanuts	10-mesh + 20-mesh	25	natural	0.92	0.496
Coconut + Peanuts	10-mesh + 10-mesh	25	denture	0.38	0.496
Coconut + Peanuts	10-mesh + 10-mesh	50	natural	0.79	0.358

As a check on the constancy of masticatory performance over a longer period of time, single tests were performed on twenty-five subjects and repeated six months later (Table I). The correlation coefficient between these two determinations was 0.92 when the calculations were based as usual on the percentage passing the 10-mesh screen. As a check on the reliability of the 10-mesh screen as a criterion, the findings were also calculated for the 20-mesh screen. It was found that the same precision was obtained when this screen was used as the criteria (Table I). The correlation coefficient between readings on the 10- and 20-mesh screen was 0.91. This indicates that the two tests were measuring the same phase of ability in mastication, and that the 20-mesh screen would provide no further information beyond that obtained with the 10-mesh screen. For this reason only the 10-mesh data have been considered.

Influence of Size of Portion.—The 15-gram sample needed for the test was arbitrarily divided into five portions because this provided a convenient amount for chewing. It seemed important to learn whether the performance would be

different if larger mouthfuls of food were masticated. The performance figures were compared on 10 half dentitions when the 15-gram sample was divided into 5, 3, and 2 portions. In each instance a total of 100 masticatory strokes were applied to the whole sample, which means that each portion was subjected to either 20, 33, or 50 masticatory strokes. The results in Table II indicate that the size of the portion does not influence the masticatory performance.

TABLE II

MASTICATORY PERFORMANCE VERSUS SIZE OF MOUTHFUL

SUBJECT	5 PORTIONS 3 GRAMS EACH 20 CHEWS	3 PORTIONS 5 GRAMS EACH 33 CHEWS	2 PORTIONS 7½ GRAMS EACH 50 CHEWS
Lou.	80.6	76.5	76.8
Man.	82.9	82.2	91.6
Bar.	76.9	75.3	75.3
Phy.	80.1	81.3	76.8
Alg.	86.7	91.1	88.8
Bru.	48.1	47.8	52.2
Ang.	97.0	98.1	98.0
Edi.	96.4	92.9	94.2
Shr.	55.3	55.7	59.8
Shl.	31.2	28.4	30.9
Average	73.4	72.6	73.9

Masticatory Performance for the Whole Mouth.—Practically all of the observations have been made on a single half dentition in order to avoid the variations that might occur if persons were allowed to use either or both sides of their dentition ad libitum. According to Ascher,[8] mastication is carried out predominantly on a single half dentition, and the chewing ability of the whole mouth is determined by the masticatory performance of the favored side. It was observed clinically during tests on several people that persons normally use only one side of their dentition in mastication. This generalization was most often true when the other half dentition had a deficient area.[21] Direct observations were still required in order to learn whether the performance figures on a half dentition would have meaning for predicting the performance of the whole mouth.

TABLE III

MASTICATORY PERFORMANCE OF WHOLE MOUTH AND EACH SIDE SEPARATELY

SUBJECT	SIDE PREFERRED	OTHER SIDE	AD LIBITUM
Bar.	76.9	71.6	71.1
Lou.	80.6	70.4	83.9
Ang.	97.0	96.4	96.4
Man.	81.5	6.0	75.0
Shi.	55.3	31.2	63.5
Yur.	90.9	92.5	93.2
Edi.	96.4	87.6	93.8
Cyn.	73.0	64.8	74.6
Lis.	96.2	87.8	96.7
Rob.	58.2	18.5	57.7
Average	80.6	62.7	80.6

This question was investigated by subjecting ten persons to masticatory performance tests on both sides of their mouths and comparing these performances to their performances when they were allowed to masticate according to

their usual habits. The results are presented in Table III. In nine cases out of ten, the side preferred in mastication was the one having the better performance; one subject (YUR) had a region in which food impacted on the side exhibiting the best performance and for this reason preferred to masticate on the other side. The average performance on the side preferred was identical with that of the mastication ad libitum. For individual cases the ad libitum performance approached that of the preferred side most closely, whenever the two sides differed by more than 6 per cent. In general, it appears that a good approximation of the masticatory performance of the whole mouth can be obtained by measuring the performance of the side that is preferred in mastication.

Shredded Coconut as a Test Material.—Shredded coconut was selected for trial as a test material requiring large chewing forces. When masticated coconut was subjected to particle size distribution analysis, it was found that its distribution was not linear when plotted in the special manner already described. With five subjects, approximately 50 to 80 per cent of the food passed through the 10-mesh screen after 20 strokes. As the number of masticatory strokes was increased to 40 and 80 chews, more food passed through the 10-mesh screen, but instead of distributing itself on the 20-, 40-, 80-, and 200-mesh screens, it merely collected upon the 20-mesh screen and failed to go any further. This fact and the lack of linearity in the particle size distribution curve cast doubt upon the ultimate reliability of shredded coconut as a test material. It was nevertheless used to obtain an indication of performance with tough foods.

Tests were performed with coconut and peanuts on several subjects having natural and artificial dentitions. The correlation coefficient between the coconut and peanut tests with twenty-five cases of natural dentition was 0.78 (Table I). This coefficient is large enough to indicate that both tests are measuring a very similar aspect of performance, and thus performance with peanuts is probably sufficient to establish the masticatory performance of the mouth. The findings were different for thirty-six denture patients. With these persons, the correlation coefficient between the two tests was only 0.38, which indicates that the two methods are measuring different aspects of mastication. Performance with peanuts is not a satisfactory indication of the denture performance with a tougher food, and two measurements are needed in order to obtain a satisfactory picture of masticatory ability.

Effect of Loss of Teeth on Masticatory Performance.—More than one hundred half dentitions have been tested for masticatory performance. Twenty-five of these were complete, fifty had third molars missing, and twenty-five lacked two molars (third and one other). In addition to these natural dentitions, thirty denture cases were studied. These groups differed considerably in their average masticatory performance. This is shown pictorially in Fig. 3. The amount of peanuts passing the 10-mesh screen after 20 chews is dependent, on the average, on the number of molars present. When all are present the performance averaged 88 per cent. For persons having the first and second molar in occlusion, the average performance was 78 per cent. Those having only a first or second molar present had an average performance of only 55 per cent, and denture wearers possessed still less ability in mastication.

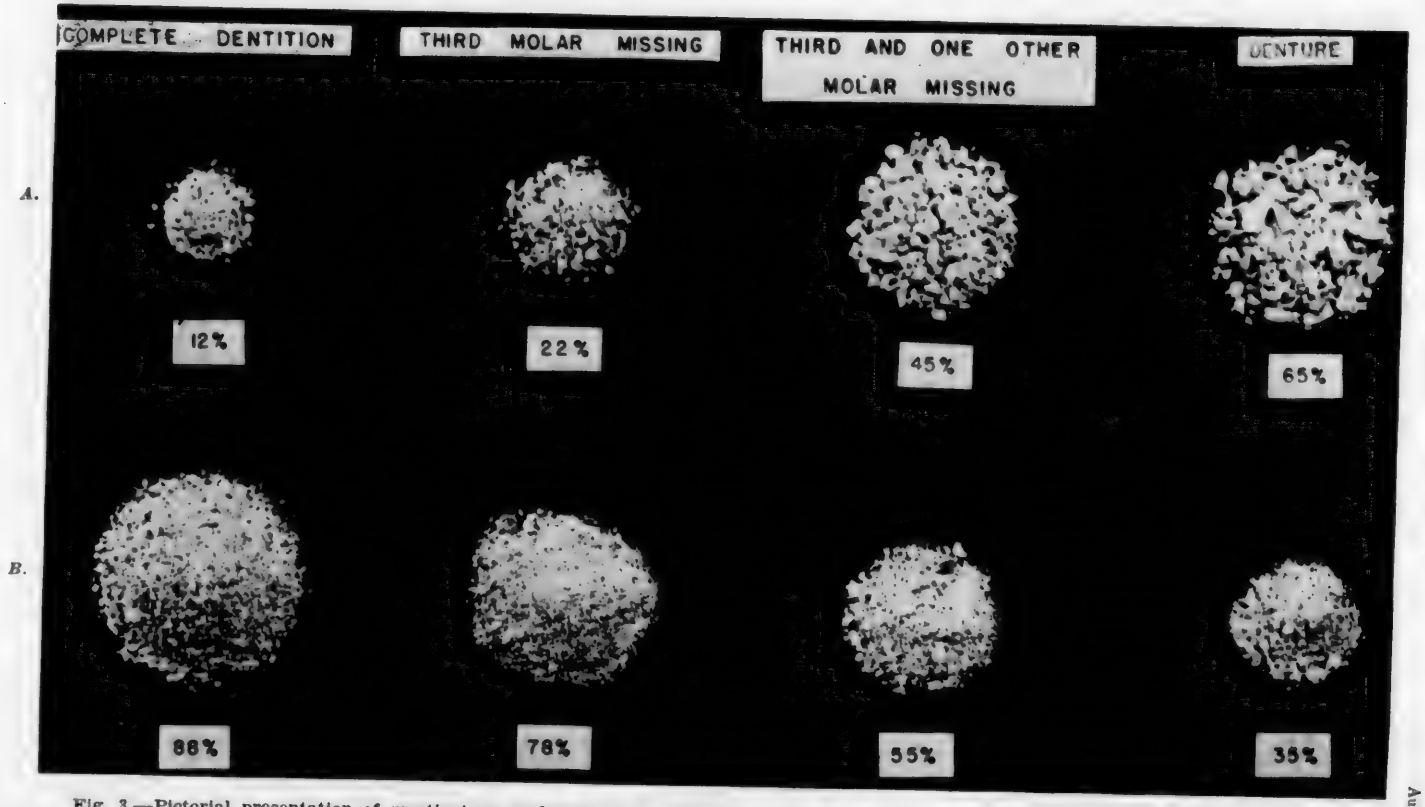

Fig. 3.—Pictorial presentation of masticatory performance of persons tested.
A, Amount of peanuts remaining on 10-mesh screen after 20 chews. *B*, Amount of peanuts passing 10-mesh screen after 20 chews.

There are wide variations in performances of individuals. This information is given in histogram form in Fig. 4. Each vertical line represents a determination on one person's half dentition. Four persons having only one molar in occlusion were able to masticate more rapidly than some persons who had a complete dentition. There were three persons with two molars in occlusion who could not chew as well as some of the denture wearers. In spite of the variation, the differences between the averages are real. This was disclosed by tests for statistical significance, which indicated that the differences were significant at the 1 per cent level.

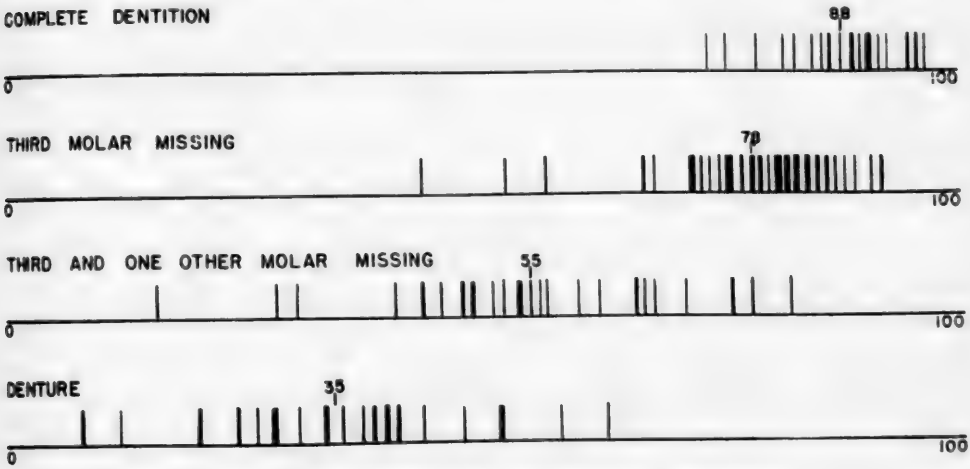

Fig. 4.—Effect of missing teeth on masticatory performance.

A similar range of variability is present when the half dentitions are grouped according to the number of tooth units present. A tooth unit has been defined as one molar pair, or two premolar pairs in occlusion.[22] Fig. 6 is a histogram presenting this information. The average performance is definitely related to the number of tooth units present, but prediction of an individual's performance is impossible on this basis. For example, among the 2 to 2.5 unit group there were six half dentitions which perform better than some of the complete dentitions, and three half dentitions which are worse than one of the 0-0.5 unit dentitions. There is so much variation in performance among persons with the same numbers of teeth that the counting of tooth units is of little value in predicting how well any individual can masticate his food.

Masticatory Efficiency.—Performance averages cannot be used directly for calculating degree of impairment of mastication. For example, the loss of third molar brings the average performance from 88 to 78 per cent, but this refers to a different size distribution of food rather than to a 10 per cent impairment of chewing faculty.

Masticatory efficiency is calculated in terms of the number of extra chewing strokes needed by the deficient person in order to achieve the same pulverization as the standard person. If the deficient person required twice

as many masticatory strokes as the normal in accomplishing this task, we would know that his efficiency was half that of the normal. The data must be converted from "percentage of food passing the 10-mesh screen" to the number of chews required to produce foods of a standard fineness.

At first an attempt was made to correlate the percentage of food passing the 10-mesh screen with the logarithm of the number of chews. This relationship was linear when the percentage of food passing the screen was between 20 and 80 per cent. Below 20 or above 80 the equation was inadequate. It became linear when the "percentage passing" was plotted on the probability scale. This is shown in Fig. 5. Here the data for ten individuals is plotted as a series of straight parallel lines within the error of the test. The heavy dotted lines in Fig. 5 indicate the averages of eight values, omitting the two extremes. It discloses that if a hypothetical average person were to chew peanuts 10 times, 44 per cent of the food would pass the 10-mesh screen; with 160 chews, 98 per cent would go through.

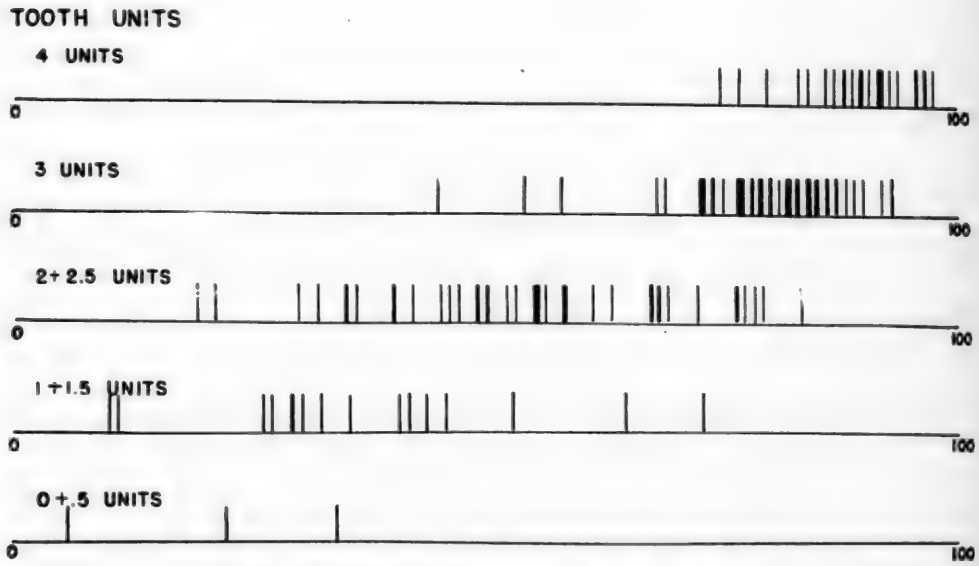

Fig. 5.—Masticatory performance versus tooth units.

The number of chews required for any desired degree of pulverization can be calculated by use of the slope of the dotted line of Fig. 5. It is illustrated in Fig. 7. The average masticatory performance of groups of persons lacking none, one, and two molars are designated by certain symbols in this figure. Since the performances were all obtained with 20 masticatory strokes, the initial points fall on a horizontal line at 20 chews. The problem was to find how many chews each of these groups would require in order to produce the same subdivision of the food. Our standard is the group of persons who have lost third molars and no other teeth, and their performance was 78 per cent.

Lines were drawn through the points for complete dentition, two molars missing, and denture groups and extended parallel with the standard slope line until they intercepted the 78 per cent line. Their points of intercept indicate that 12 strokes would be required for the complete dentition, 46 for two molars missing, and 86 for denture wearers. Efficiencies of the groups were calculated by dividing these values into 20. The efficiencies were 166 per cent, 44 per cent and 23 per cent, respectively. The calculated effect for the third molar

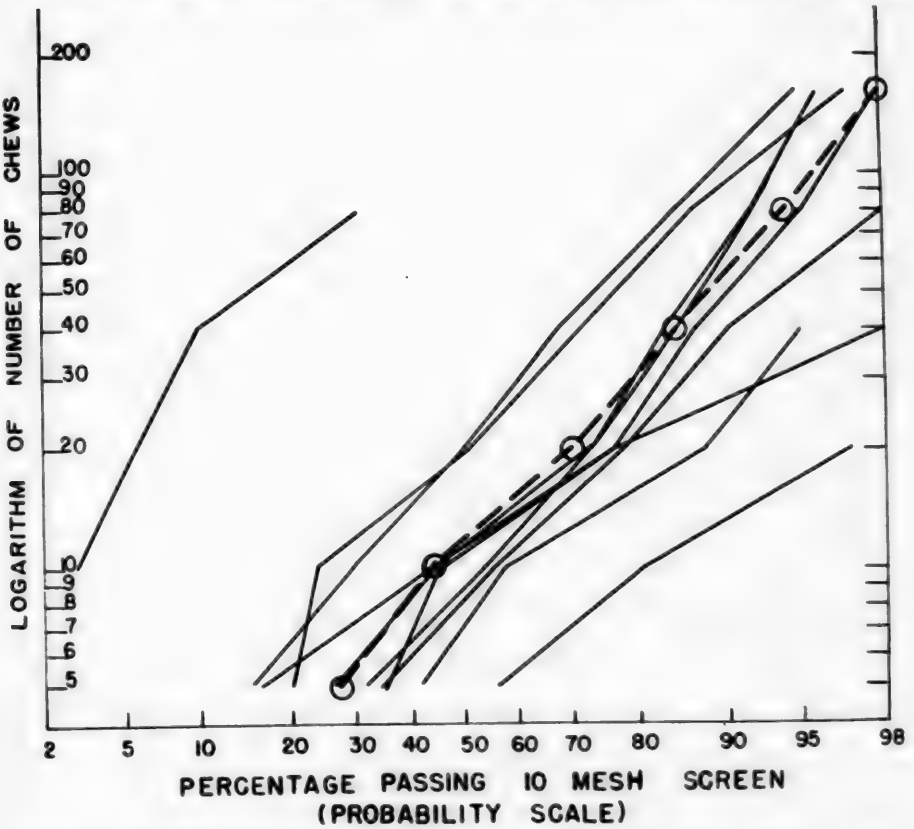

Fig. 6.—Percentage of food passing 10-mesh screen versus logarithm of number of chews.

appears to be too high, but the other two values seem to be reasonable. It is realized that these efficiencies should be considered tentative until they have been checked by having suitable numbers of persons actually produce food with the standard degree of pulverization. The number of masticatory strokes required to achieve such pulverization would be measured and converted to efficiencies. The studies should also include comparisons between groups with tougher foods.

This calculation can be compared with the findings of one other investigator. Dahlberg's mastication coefficient for men with extremely good teeth was 16,

and was merely reduced to 12 for the group with extremely poor teeth. This might indicate that the performance of a poor dentition was merely 25 per cent less than that for a good dentition were it not for calculations which can be made from the data on page 126 of his monograph.[13] Apparently 56 masticatory strokes would be needed by persons with poor dentition in order for them to obtain the same masticatory coefficient as the good dentition group. The latter used 20 chews for their coefficient, which would suggest that average efficiency of the poor dentition group was actually 36 per cent of the good dentition group.

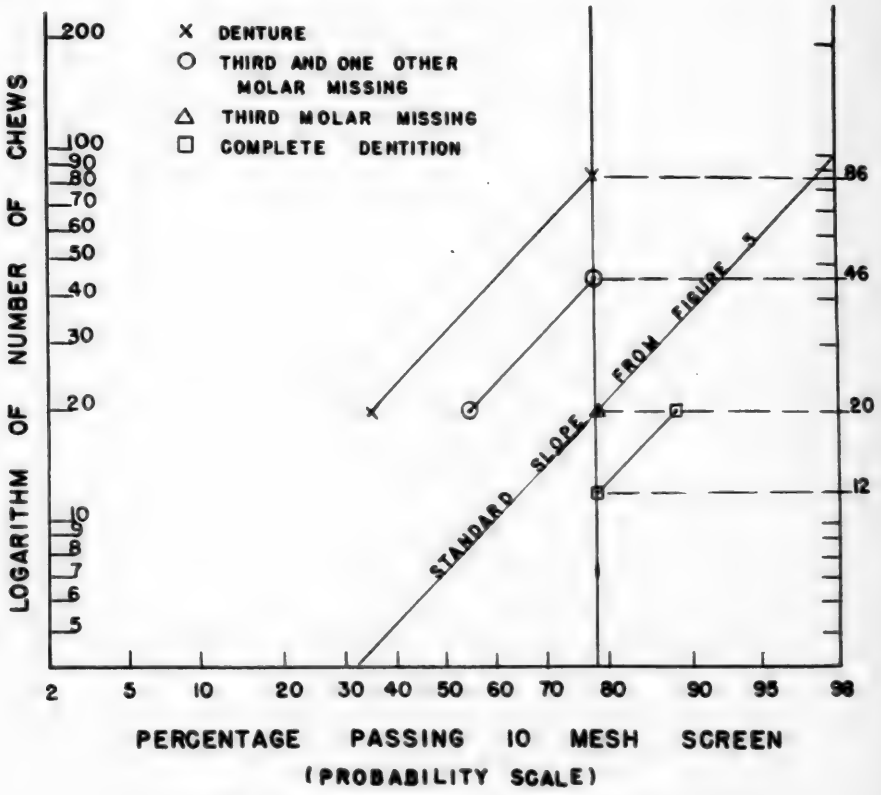

Fig. 7.—Method of calculating average efficiency.

The values of performance herein reported cannot be compared with those of previous workers. In many instances different foods were used and larger or smaller screen openings were employed as the criterion for performance. Ascher[8] discarded particles finer than 0.3 mm. and Dahlberg[13] those smaller than 1.0 mm. In our experience with peanuts, even the fraction which passes a 200-mesh screen must be weighed in order to have the data obey the logarithmic probability rule. Dahlberg actually found very few particles which collected

on a 0.5 mm. screen, but it is possible that the fine particles of gelatin which were employed were dissolved during the excessive washings that were used. Another author[9] did not determine the total weight of food recovered, and thus subjected his findings to error because of food swallowed or retained in the mouth.

Loss in masticatory efficiency is apparently not compensated by longer mastication time as demonstrated by Dahlberg[13] (p. 135). It is common knowledge that persons with dentures do not use four times as long to consume their food as do those with a full dental complement. The actual compensations employed are either adjustment of the swallowing habit to permit ingestion of larger particles or avoidance of foods requiring mastication. Perhaps a change in food preferences would lead to omission of tough foods[23] from the diet. Many such foods have a high vitamin and essential amino acid content, and failure to consume them would increase the risk of dietary deficiency.[24] Thus, the effect on food selection rather than upon absorption may be the more important systemic influence of defective dentitions.

SUMMARY

A method has been devised for measuring masticatory performance and efficiency. The masticatory performance was based on the percentage of masticated peanuts which would pass through a 10-mesh screen after being subjected to 20 masticatory strokes. Efficiency was calculated from the number of chews required to reach a desired degree of food pulverization. The performance was found to be independent of the size of mouthful as long as the number of chews was kept constant, and the chewing performance of uninstructed subjects approached that obtained when mastication was confined to their best half dentition.

The particle size distribution has been determined for masticated peanuts which had been chewed for 5, 10, 20, 40, 80 and 160 strokes, by each of ten subjects. The distribution was linear for food chewed five times when the data were plotted in a special manner. This indicated that the grinding was random in nature. As mastication proceeded, the process gradually became selective, with the larger particles disappearing more rapidly than would be expected on the basis of random grinding.

The percentage of masticated peanuts passing the 10-mesh screen was proportional to the number of chews when the data were plotted on logarithmic probability paper. This formed the basis for calculating the number of masticatory strokes required to produce a standard degree of pulverization, and from this, masticatory efficiency.

The average performance of twenty-five cases with complete dentitions was 88 per cent. Fifty half dentitions lacking the third molar averaged 78 per cent. Twenty-five half dentitions which possess two premolars and one molar in occlusion had a mean performance of 55 per cent, and thirty denture cases averaged 35 per cent. The efficiencies of the four groups are tentatively calculated to be 166, 100, 44, and 23 per cent, respectively.

The authors wish to acknowledge advice and encouragement from the late Dr. John T. O'Rourke during the early phases of the study.

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ELECTRICAL ACTION CURRENTS DURING MASTICATION

Measurement of the Effort Exerted in Chewing Various Foods

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INVESTIGATION in the field of bite strength were limited in the past to experiments measuring the maximum biting force of a tooth or denture and to calculations based on measurements of muscle cross sections on anatomical specimens.[1]

The principles on which the different instruments are built vary; some of them use a mechanical device on the lever principle, some are electrical,[2] all are inserted between the teeth and are useful in measuring maximum biting strength, while they interfere with the ordinary chewing action in the mastication of different foods. A review of the reported methods was compiled by Brawley and Sedgwick.[3] The force of mastication required ordinarily varies widely and is usually far from the maximum force expendable. Klatsky[4] made a cinefluorographic study of the jaw action in mastication of various foods, but this method is subjective and does not lend itself to measurements. None of the mentioned methods performed with the gnathodynamometer and similar instruments allows for analysis of various single foods and their chewing requisites.

Dahlberg[5] observed the number of chewing motions when consuming food. These measurements are not in direct rapport to the functional effort except that vigorous chewing may very considerably diminish the number of chewing motions necessary. Soft bread or, in the extreme case, chewing gum may require a great number of chewing motions and a minimum of functional effort. In most foods this functional stress and strain is small, in others of tougher texture the effort exerted in the same number of chewing motions may be a multiple of the force used in eating softer food.

THE ELECTROMASTAKOGRAM

In the search for a sensitive method which would indicate differential values measurable on various foods, the electromyogram was selected as a practical and expedient technical procedure. The output of electrical energy during the process of mastication can approach or exceed 1 millivolt. If recorded with a sensitive string galvanometer it provides an invaluable guide to study the range of function of the masticatory apparatus and it offers at the same time, indirectly, an index of the actual comparative pressure exerted by the teeth, regardless of the possible maximum biting strength which is more suitably measured with the previously mentioned devices.

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The contraction of the masticatory muscles involves a change in electrical potential and sets up a wave of electrical disturbance which represents a diphasic action current. In the present experiment the oscillations of the spike potential were photographed during the chewing of various foods. The current was derived from two electrodes of which one was placed squarely over one masseter muscle, and the second to the center of the forehead, just below the hairline. Lead cables were attached to the electrodes and connected with a string galvanometer. The galvanometer was calibrated so that a deflection of 1.0 cm. indicated a change in electrical potential of 1 millivolt. For the tracings, electrocardiographic paper was used, the vertical ruling indicating a time interval of 0.2 second, between any two heavy vertical time-marking lines, and the interval between any two light vertical lines measured 0.04 second.

A number of representative foods were used in the experiment and the recorded electromyogram values are derived from measurements taken on myogram tracings from young adult males. The accompanying photographs represent the tracings closest to the average findings. This highly sensitive method pictures the masticatory value of various foods which are otherwise difficult to compare objectively. To obtain a uniform basis for a comparison the oscillations during the chewing action only were recorded, omitting the initial biting act with the incisors. At the selected level of sensitivity, secretory and nervous functions did not create any noticeable interference and the electrical disturbance from the act of deglutition was eliminated by appropriate placing of the electrodes.

The muscles concerned in the act of mastication are essentially the masseter, temporal, and internal pterygoids, while the muscles at the bottom of the oral cavity as the digastric, the mylohyoid, and the geniohyoid, depress the jaw, a function not varying in effort. The lateral movements of the jaws are produced by the external pterygoids if they act separately and their simultaneous contraction causes projection of the lower jaw. The grinding movements during the act of mastication of food are produced by a combination of the masseter, temporal, and pterygoid muscles. The action current of these muscles is best derived by the described placing of the electrodes. The action current of the tongue muscles is derived from a lead placed at the suprahyoid triangle and, as the action of the tongue muscle serves primarily to keep the food properly placed for the action of the teeth and to gather it into position for the act of swallowing, it is timed predominantly between contractions of the major masticatory muscles and is not recorded on these tracings.

CHEWING EFFORT WITH VARIOUS FOODS

The following list of foods is tabulated in the order of their maximum sustained deviations, expressed in millivolts whereby the positive and negative wave of the diphasic current were added.

AVERAGE SUSTAINED ACTION CURRENT ABSENT OR MINIMAL

Fluids, including juices, beverages, and soups
Fish, cooked
Eggs, hard boiled
Cheese
Rice, cooked soft
Farina
Puddings
Ice cream
Carrots, cooked
Peas, cooked
Potatoes, cooked
Bananas
Berries
Cantaloupe
Oranges
Peaches

AVERAGE SUSTAINED ACTION CURRENT BELOW 0.3 MILLIVOLT

Beef, boiled
Bacon
Poultry, fried
Pastry
Spaghetti, undercooked
Apples, raw
Radish
Factory bread

AVERAGE SUSTAINED ACTION CURRENT BELOW 0.5 MILLIVOLT

Beef, round steak
Nuts
Carrots, raw
Toast
Hard biscuits
Beef liver, fried
Shredded wheat
Lettuce
Peppers, raw
Chewing gum

AVERAGE SUSTAINED ACTION CURRENT BELOW 0.8 MILLIVOLT

Bread crust, well-baked rye bread, 2 days old
Rice, cooked less than 5 minutes
Fresh coconut meat, mature nuts

AVERAGE SUSTAINED ACTION CURRENT BELOW 1.0 MILLIVOLT

Bread, European peasant style (of a type edible many weeks after baking) 5 days old

AVERAGE SUSTAINED ACTION CURRENT BELOW 1.2 MILLIVOLTS

Sugar cane

COMMENTS

Few foods of high masticatory value are commonly available. Those occasionally considered to provide function for the teeth, as raw carrots or celery, toast, biscuit, chewing gum, provide for relatively little or for very short and nonsustained function. In some food items the manner of preparation is all important, as in bread and rice. Of the highest functional value are bread crust of well-baked bread, undercooked rice, meat of ripe coconuts, and, particularly, sugar cane.

Most of the modern staple foods require little mastication not from the angle of duration but of intensity of the chewing action. In previous papers[6] this fact has been brought in causal relationship to a possible regressive process in the protoplasmatic component of enamel and dentin, involving a change from immunity to susceptibility to bacterial invasion, and leading ultimately to dental caries.

Figs. 1 to 6.—Galvanometric tracings during mastication of some representative foods.

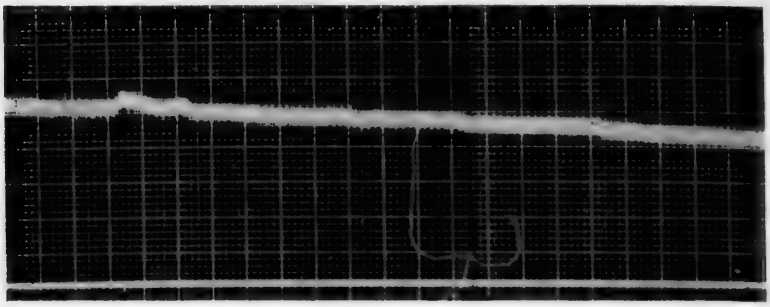

Fig. 1.—Farina.

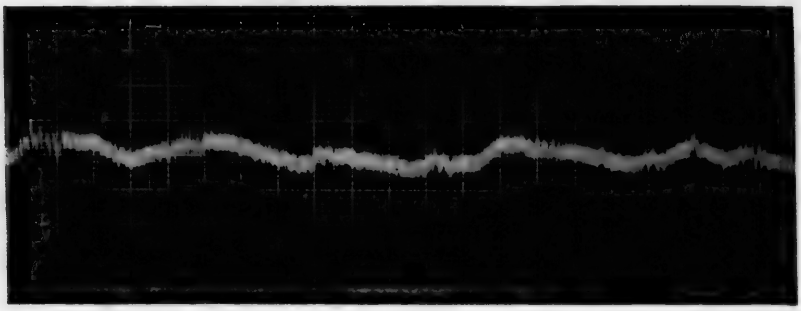

Fig. 2.—Factory bread.

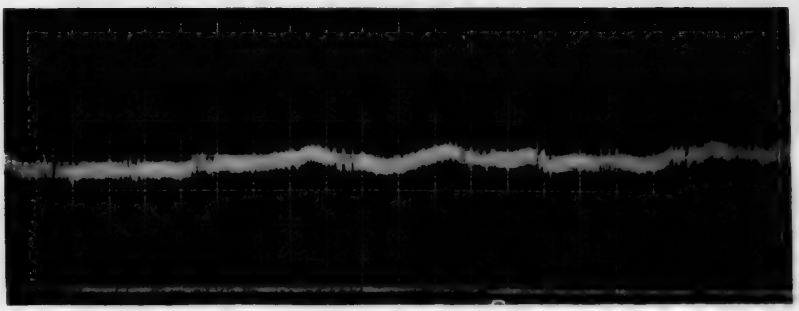

Fig. 3.—Raw carrots.

Fig. 4.—Fresh coconut meat, mature nuts.

It is important to recognize that history taking regarding chewing habits is generally worthless, as most individuals consider that they do a "normal" amount of chewing regardless of extreme differences in their food selection, preparation, and table manners, such as cutting of food or habitual size of bite. English college students on a notoriously soft diet requiring minimal mastication, as well as South Pacific aborigines, making considerable use of their teeth and jaws, expressed their opinions regarding their chewing habits as being the "normal amount." Any investigation of masticatory function and food selection from this angle as well as an attempt of correlating caries incidence and dental underfunction should therefore rely on objective analysis and measurement.

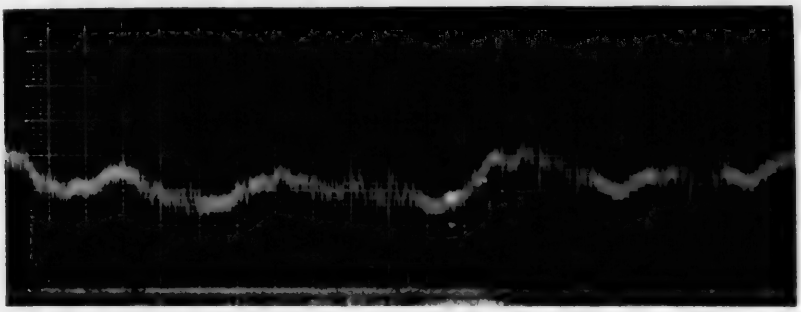

Fig. 5.—Tough bread crust, well-baked bread 5 days old. European peasant type bread, edible for weeks after being baked.

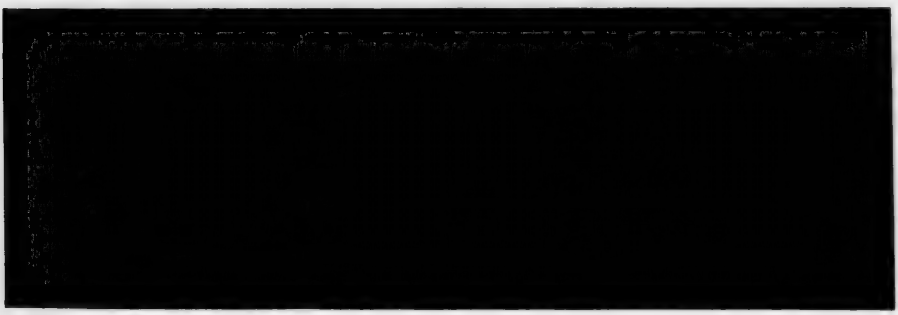

Fig. 6.—Sugar cane.
*The oscillations of the base line are due to contractions of the sternocleidomastoid muscle with lateral movements of the head and should be disregarded.

SUMMARY

By means of the electromyogram, the energy produced during chewing was measured. On photographs of the action currents, the amplitude of oscillation was read directly in millivolts.

Differences in chewing habits can generally not be established by history taking. Most individuals consider that they chew a normal amount, though

the vigor of mastication varies extremely. Objective analysis and measurement can portray the chewing habits and the functional values of various foods.

Most ordinarily available foods require a minimal amount of mastication before deglutition. The foods that require more mastication were found to be well-baked bread crust, undercooked rice, mature coconut meat, and, particularly, sugar cane. Contrary to common belief, toast, biscuit, chewing gum, raw vegetables, and fruits require little chewing if the vigor and not duration of mastication is under scrutiny.

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HYGROSCOPIC EXPANSION OF SOME CASTING INVESTMENTS

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CREDIT is generally given to Taggart[1] for introducing the cast gold inlay to the dental profession. Although he realized that gold shrinks when cooled from molten state to solid state of room temperature, the investment available to him did not supply sufficient compensation. In 1910, Van Horn[2] published a paper dealing with a method by which he obtained the compensation by means of wax expansion. The next major research in connection with this problem was the work of Coleman[3] at the National Bureau of Standards. He determined the gold shrinkage to be 1.25 per cent and presented a casting technic with compensation values of quantitative nature. The total compensation for the gold shrinkage was obtained by the combination of the setting and thermal expansion of the mold. This procedure is the general practice followed at the present time for compensation.

There are some disadvantages to this method. Skinner has indicated the difficulty of obtaining smooth castings and the improper compensation for the casting shrinkage.[4] It has been found by Hollenback[5] and Scheu[6a] that castings made at a high temperature are rougher than those made at a low temperature (450° C.), depending upon the great difference in thermal expansion between plaster of Paris and silica, which causes a poor union of the fine particles. This may also result in friability and there may be some warpage due to the differential expansion. A method which provides sufficient compensation at a low mold temperature is the hygroscopic casting technic.[6b]

Wilson[7] showed that certain plasters expand as much as 0.0044 inch to the inch by taking up moisture from the air. With this information at hand, Scheu[6a, b] tested some commercial investment to determine what results the addition of moisture would have. He found[6b]: (1) All of the casting investments that were tested expanded. (2) The thicker the mix, the greater the expansion. (3) A mix with hot water expanded more than one with cold water. (4) The time at which the mix is placed in the water is of great importance. It was found that if the mix is placed in the water early, it would not expand so much as when placed in a little later. It was also found that if the mix was placed in the water after a certain time, it could not expand to the maximal amount. (5) The temperature of the water in which the mix is placed is important, for two reasons; (a) the temperature of the water controls slightly the expansion of the investment, and (b) water at a temperature of 100° F. slightly softens the wax pattern so that the fine margins are not marred while expansion is taking place. The mix should remain in the water at least 20 minutes to obtain the full expansion, after which time no further expansion is obtained.

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Docking[8] found that both a high silica content and fine silica particles gave increased hygroscopic expansion. The use of plaster of Paris instead of hydrocal reduces the hygroscopic expansion to such an extent that mixtures of plaster and silica may be unsuitable to this new technic. Both a thick mix and warm water give increased expansion. Like normal setting expansion, the hygroscopic type is very sensitive to restrictive forces such as those provided by the interior surface of the ring or by a tightly packed lining. The amount of water taken up will affect the ultimate expansion and to get full expansion the invested pattern is completely immersed in water and left for a period of time. According to Docking, most of the expansion takes place in the first 10 to 12 minutes and there is little further expansion after about 45 minutes. The temperature of the bath was maintained at 100° F., which eliminates the need for compensation of shrinkage of the wax pattern from oral to room temperature. This temperature also helps to soften the wax slightly in order to reduce its interference with the expansion. With regard to the temperature of the bath, Docking observed the same result as Scheu. According to Docking, "The hygroscopic technique is recommended for general use only if a thorough study is first made of it in relation to the particular investment to be applied."

In his investigation, Degni[9] found that hygroscopic expansion is very sensitive to confinement of any kind. He also found that the greater the amount of hydrocal in the investment, the less will be the hygroscopic expansion. While the mechanism of hygroscopic expansion is not completely known, a colloidal theory for the setting of plaster of Paris is frequently advanced,[10] and Degni used this to explain hygroscopic expansion.

METHODS FOR MEASURING EXPANSION

Several methods have been used for measuring hygroscopic expansion. Coleman,[11] at the Bureau of Standards, used a V-shaped support for the investment and two micrometer microscopes attached to a common base. The investment was mixed and poured into the V-shaped support, which was lined with waxed paper to prevent the investment from adhering to its walls. A small metal plate was placed at each end of the sample. The micrometer microscopes were focused on reference lines which were ruled on the plates and the length change between the reference lines were measured with the microscope. Although Coleman used this method to measure normal setting expansion, it has been used later by some investigators to study hygroscopic expansion.

In Scheu's experiments, a mix of investment was placed in a rectangular mold formed with precision gauge blocks on a glass slab. The investment with gauge block mold was immersed in water after the initial set.

Docking[8] used an entirely different method where he allowed the investment to float in a bath of mercury. Here the specimen was quite free to expand or contract against practically no external forces. After addition of water, readings are made with a micrometer microscope focused on a needle placed in the investment. In a later study Docking used a modification of the inlay ring method employed by Degni.

Degni reported having made a number of trials with the Coleman method but could not obtain the values reported by Scheu (1.6 per cent) and abandoned the metal trough as unsuitable for measuring hygroscopic expansion depending upon the friction between the liners in the metal trough and the investment. He then applied a dial gauge method. A glass bowl with electrically heated water was used. The specimen was prepared in a casting ring with wet asbestos lining. A thin glass plate, made from a portion of a microscope cover slide, was placed on the top of the specimen. A dial gauge graduated in 0.01 mm. was applied on the glass slide and readings were taken each minute. Some of the specimens were prepared in a wax ring which were withdrawn when the mix had thickened sufficiently and the specimen was immersed in water. The hygroscopic expansion could be measured in that way. Watts[12] used the dial gauge method in determining the hygroscopic expansion due to one layer of wet asbestos, which he found to be 0.16 per cent.

After studying the results obtained by different investigators, it is evident that they vary with the method of measuring. With the mercury bath, Docking measured the dimensional changes which take place while the mixture is still semifluid and adaptable. "These would be normally taken up as volume changes in the mix which in the initial stages is too weak to expand or compress a wax pattern or otherwise to alter the dimension of the cavity occupied by the pattern. Any expanding forces, for instance, attempting to draw the investment away from the pattern, will be insufficient to overcome the action of gravity which forces the semifluid mix to conform to the contours of the wax. From the practical point of view, therefore, it is the expansion after the mix has set enough to support itself that is important."[8] The stage at which this condition occurs is obscure. The setting time obtained by a Gillmore needle is not necessarily the time at which the investment will show effective expansion. The arbitrary nature of setting time and expansion determination must, therefore, always be kept in mind. In his work with determination of normal setting expansion of investments, Skinner[13] has stated that any changes in dimension before the initial set are of no practical importance, but that is not necessarily true in the hygroscopic expansion of the investments.

PURPOSE AND METHOD OF MEASUREMENT

The purpose of this investigation was to study the hygroscopic expansion of some of the investments generally in use at the present time. It was desired to study them under conditions most nearly approaching those occurring in dental practice; therefore, the dial gauge method of Degni was chosen, where the specimen is prepared in a casting ring with wet asbestos lining. A weighed quantity of investment powder was added to a measured amount of distilled water at room temperature in the mixing bowl. The mix was then spatulated by hand for 30 seconds and poured into the casting ring lined with wet asbestos. On top of the ring a microscope cover slide was placed with a length equal to the diameter of the ring. The specimen was then placed in a water bath kept at a constant temperature (±2° F.) by means of a Bunsen burner, and the

temperature was observed by a thermometer inserted in the bath. The expansion of the specimen was measured by the dial micrometer placed in contact with the microscope cover slide on top of the specimen. In order to reduce the pressure of the dial gauge on the specimen, the dial gauge was counterbalanced. A thread was attached to the head of the gauge spindle and then passed over a pulley with a weight of 36 Gm. suspended at its free end. The apparatus is shown in Fig. 1. The test samples had an initial length of 1.493 inches.

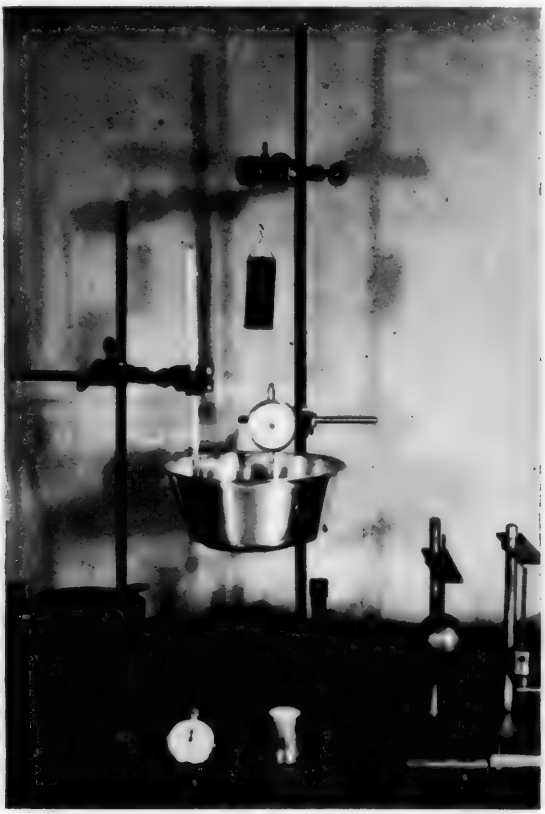

Fig. 1.—Apparatus for measuring hygroscopic expansion.

Some of the trials were carried out in the same way as in Scheu's[6b] investigations, where the investment was allowed to set in air before being immersed in the water bath. Other experiments were made with immersion of the specimen in water at different times before setting. The initial set was determined by a Gillmore needle. The water-powder ratio also was varied in order to study how that would affect the expansion. The temperature of the water bath was in most cases kept at 37° C., but in some it was raised to 50° C. in order to investigate the effect of the temperature on the expansion. There were also some experiments where two asbestos linings were used instead of

one. In each experiment, readings were taken at 1-minute intervals in the early stages and then at 5-minute intervals toward the end of the measurement period, which was 60 minutes for each sample.

The following investments were studied:

Name	*Manufacturer*
Hygroscopic Investment	Ransom and Randolph Company, Toledo, Ohio
Gray Investment	Ransom and Randolph Company, Toledo, Ohio
Beauty Cast Investment	Whip-mix Corporation, Louisville, Ky.
Cristobalite Investment	Kerr Manufacturing Company, Detroit, Mich.

TABLE I

RANSOM AND RANDOLPH HYGROSCOPIC INVESTMENT

CURVE	WATER-POWDER RATIO	TEMP. OF WATER BATH (° C.)	INITIAL SET (MIN.)	TIME OF IMMERSION (MIN.)	MAXIMUM EXPANSION (%)
A					
1	0.31	37	16	immediate	1.88
2	0.31	37	14	14	1.41
3	0.31	37	16	16	1.21
4	0.31	37	18	18	1.02
5	0.31	37	18	20	0.87
6	0.31	37	18	30	0.33
B					
1	0.31	50	17	16	1.44
2	0.31	50	16	16	1.42
3	0.31	37	16	16	1.24
4	0.31	37	16	16	1.21
C					
1	0.31	50	18	18	1.21
2	0.31	50	18	18	1.12
3	0.31	37	18	18	1.04
4	0.31	37	18	18	1.02
D					
1	0.31	37	18	14	1.84
2	0.31	37	18	14	1.77
3	0.31	50	18	14	1.76
4	0.31	50	17	14	1.74
E					
1	0.31	37	17	18	1.14
2	0.31	37	17	18	1.12
3	0.31	37	18	18	1.04
4	0.31	37	18	18	1.02
F					
1	0.28	37	15	immediate	2.02
2	0.34	37	19	immediate	1.93
3	0.30	37	16	immediate	1.93
4	0.31	37	18	8	1.93
5	0.33	37	19	immediate	1.91
6	0.31	37	17	immediate	1.89
7	0.31	50	16	immediate	1.88
8	0.31	37	16	12	1.88
9	0.32	37	18	immediate	1.85
10	0.31	37	16	immediate	1.85
11	0.36	37	20	immediate	1.82

EXPERIMENTAL DATA

The results from various test conditions are plotted in graphs representing the total data taken for each condition of test. In each graph, the time in

minutes is plotted on the base of the graph with the expansion in 1/1000 inch on the left and corresponding percentage on the right. The first six graphs represent results obtained from tests under different conditions on Hygro-

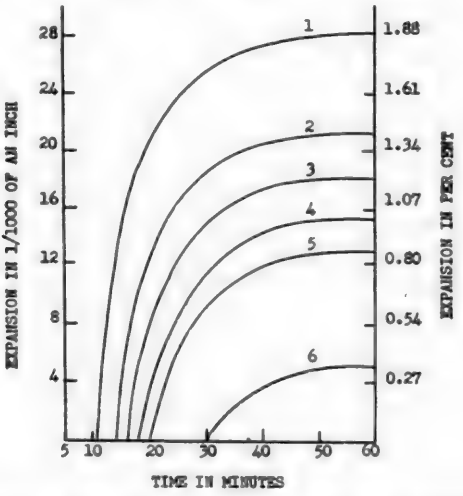

Fig. 2.—Ransom-Randolph Hygroscopic Investment. Effect on expansion of time between mixing and immersion.

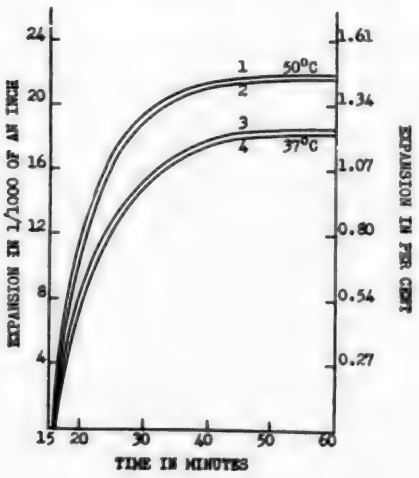

Fig. 3.

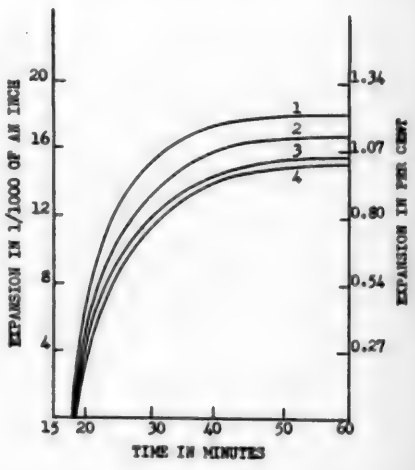

Fig. 4.

Fig. 3.—Ransom-Randolph Hygroscopic Investment. Effect on expansion of temperature of the water bath. Immersed 16 minutes after mixing.

Fig. 4.—Ransom-Randolph Hygroscopic Investment. Effect on expansion of temperature of the water bath. Immersed 18 minutes after mixing.

scopic Investment. A summary of test conditions and the maximum expansion obtained for each test condition is given in Table I which corresponds to data in the graphs of Figs. 2 to 7.

In Fig. 2, Curve 1 was obtained when immersion in the water bath was made in 2 minutes, or as soon as possible after mixing, and shows the greatest expansion. Although immersed promptly after mixing, the investment did not possess sufficient strength to develop a measurable change in dimension until 11 minutes after mixing. Curves 2, 3, and 4 were obtained when immersion was made at the time for initial set, determined with a Gillmore needle. Curve 2 was obtained when the investment was taken from a newly opened can and had a setting time of 14 minutes. Some days later the setting time was not the same, even though the same powder-water ratio and other test conditions were used. The immersion was made after an initial set of 16 minutes (Curve 3) and the expansion was less. A week later the setting time was 18

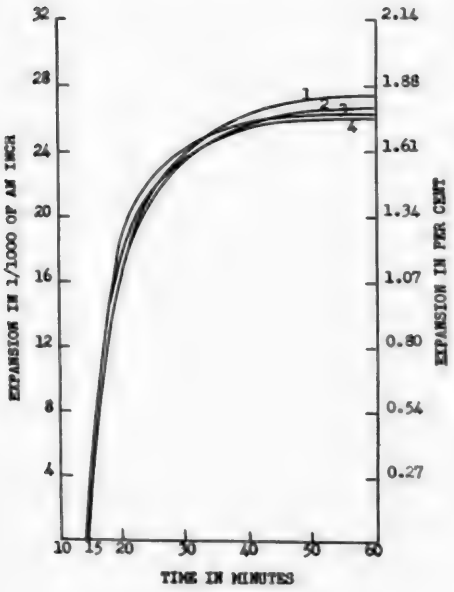

Fig. 5.

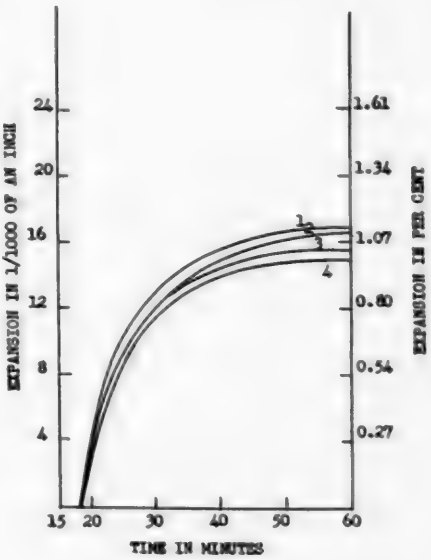

Fig. 6.

Fig. 5.—Ransom-Randolph Hygroscopic Investment. Effect on expansion of temperature of the water bath. Immersed 16 minutes after mixing.

Fig. 6.—Ransom-Randolph Hygroscopic Investment. Effect on expansion of two asbestos linings.

minutes, and the values for expansion are seen in Curve 4. The change in initial setting time perhaps was due to moisture taken up by the investment from the air. Results shown by Curves 5 and 6 were obtained when immersion was made a certain time after initial set, which was 20 and 30 minutes after mixing. The results in Fig. 2 show that the hygroscopic expansion is greatest when the specimen is immersed immediately after mixing, and the expansion decreases with increased time between mixing and immersion in water.

In Fig. 3 and Table I (B) are given the results obtained when immersion was made at the time of the initial set, which in this case was 16 minutes after

mixing. Values in Curves 1 and 2 were obtained when the temperature of the water bath was 50° C., and Curves 3 and 4 represent a water-bath temperature of 37° C. It is seen that there is a slight increase in expansion (0.2 per cent) with increased temperature of the bath.

When immersion was made 18 minutes after mixing (Fig. 4, Table I [C]), which was also the initial setting time for this series of samples, a water bath temperature of 50° C. gave values shown in Curves 1 and 2. Values in Curves 3 and 4 were obtained when the investment was immersed in water at 37° C.

In Fig. 5 and Table I (D) are given the values obtained when immersion was made 14 minutes after mixing but 3 to 4 minutes before the initial set. A water-bath temperature of 50° C. was used to obtain values shown by Curves 1 and 2, with Curves 3 and 4 representing values obtained from a bath of 37° C. It will be observed that the curves are rather close together and the temperature does not seem to affect the expansion appreciably when the immersion is made before the initial setting time.

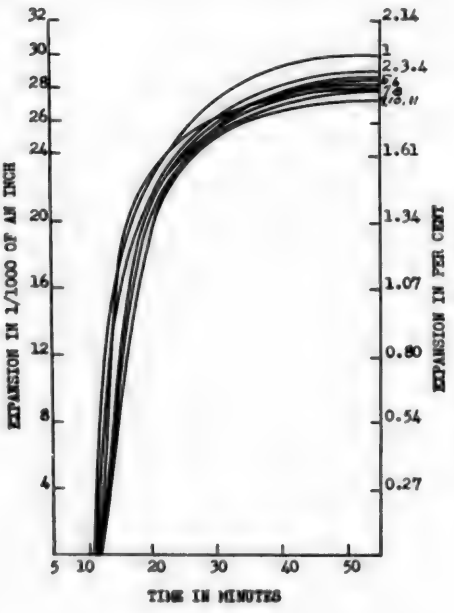

Fig. 7.

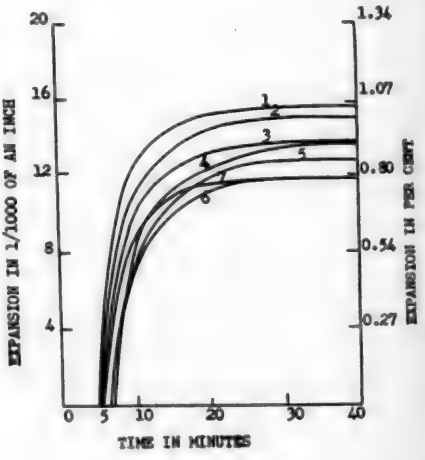

Fig. 8.

Fig. 7.—Ransom-Randolph Hygroscopic Investment. Expansion when immersion is made before initial set with variations in water-powder ratio and in temperature of the water bath.

Fig. 8.—Ransom-Randolph Gray Investment. Expansion of Gray Investment when immersed immediately and after initial set.

The results shown in Fig. 6 and Table I (E) indicate a slight increase in the expansion (0.1 per cent) when two asbestos liners are used (Curves 1 and 2) in the ring instead of one. In this series of tests, the specimens were immersed in a 37° C. water bath at the time of initial set, which was 18 minutes after mixing.

The curves shown in Fig. 7, with corresponding data in Table I (F), were obtained when immersion was made before the initial set. The experiments were made with the mix in a semifluid consistency when immersed. Results in Curve 1 represent a thick mix with water-powder ratio of 0.28, while Curve 11 represents a thin mix with water-powder ratio of 0.36. These two curves indicate a tendency toward increased expansion when a thick mix is used. If Curves 1 and 11 are not considered, the remainder of the curves are within a very narrow range, in spite of variations in water-powder ratio between 0.30 and 0.34 and water bath temperatures between 37 and 50° C. Even though the test specimens were immersed in the water bath at intervals from 2 to 12 minutes after mixing, almost the same hygroscopic expansion occurred. As seen from these curves, the variation in expansion for this product is so small as to be of little practical significance when limited variations are made in the water-powder ratio, water-bath temperature, and time of immersion before the initial set. This is thought to be a good quality, since in practice some types of patterns require longer investing times than others.

The data shown in Fig. 8 and Table II represent values obtained when Gray Investment is used. This investment has been designed for use with thermal expansion technics. The expansion is slightly greater when immersion is made immediately after mixing (Curves 1, 2, and 3) than when immersed after the initial set (Curves 4, 5, and 6). The low value shown by Curve 7 may be the result of the confinement of the cover slide during the experiment. The hygroscopic expansion of this investment is rather low and not sufficient for full compensation of the gold shrinkage. In an investment designed for thermal expansion, a low hygroscopic expansion should be considered a good quality; otherwise, the use of wet asbestos liners may be hazardous.

TABLE II

GRAY INVESTMENT

CURVE	WATER-POWDER RATIO	TEMP. OF WATER BATH (° C.)	INITIAL SET (MIN.)	TIME OF IMMERSION (MIN.)	MAXIMUM EXPANSION (%)
1	0.29	37	6	immediate	1.04
2	0.29	37	7	immediate	1.00
3	0.29	37	6	immediate	0.92
4	0.29	37	7	7	0.90
5	0.29	37	7	7	0.85
6	0.29	37	6	6	0.79
7	0.29	37	7	immediate	0.79

When Beauty Cast Investment was used, results of tests were as shown in Figs. 9 and 10, Table III (A and B). In Fig. 9, it is seen from Curves 2, 3, 4, and 5 that the expansion decreases as the time between mixing and immersion increases. Results indicated by Curve 1 show that a maximum expansion in this investment may be obtained when the specimen is immersed at a definite time after mixing but before the initial set. In this case, the specimen was immersed 15 minutes after mixing but 3 minutes before initial set. Values in Curve 3 were also obtained when the specimen was immersed after 15 min-

utes, but in this case the investment had set before immersion. The relatively great hygroscopic expansion represented by Curve 1 corresponds to an observation made by Scheu that some investments show the maximum expansion when immersed at a certain time after mixing but before the initial set has taken place.

TABLE III

BEAUTY CAST INVESTMENT

CURVE	WATER-POWDER RATIO	TEMP. OF WATER BATH (° C.)	INITIAL SET (MIN.)	TIME OF IMMERSION (MIN.)	MAXIMUM EXPANSION (%)
A					
1	0.30	37	18	15	2.31
2	0.30	37	18	immediate	2.04
3	0.30	37	15	15	1.57
4	0.30	37	17	17	1.39
5	0.30	37	18	25	0.62
B					
1	0.30	37	18	15	2.31
2	0.30	37	17	10	2.06
3	0.28	37	15	immediate	2.04
4	0.30	37	18	immediate	2.04
5	0.30	37	17	immediate	2.03
6	0.30	37	17	7	1.93
7	0.30	37	17	14	1.89
8	0.30	37	18	immediate	1.86
9	0.30	50	17	immediate	1.83
10	0.30	37	17	immediate	1.81
11	0.30	50	17	immediate	1.69
12	0.32	37	18	immediate	1.68

The results shown in the curves of Fig. 10 were all obtained when immersion was made before initial set. Curve 1 in Fig. 10 is the same as Curve 1 in Fig. 9, while Curve 2 resulted from a thick mix with water-powder ratio 0.28 and Curve 12 from a thin mix using a water-powder ratio of 0.32. The expansion increases with the thickness of the mix, although the results indicate a lack of uniform results under the same conditions of test. Immersion immediately after mixing when using similar conditions for water-powder ratio and water-bath temperature gave varying results between 1.81 and 2.04 per cent expansion. Although the variations in investing and test conditions were no greater than those that may occur in laboratory practice, the expansion results, shown in Fig. 10, vary between 1.69 and 2.31 per cent.

The values presented in Fig. 11 and Table IV (A) for Cristobalite Investment show that the hygroscopic expansion increases with the thickness of the mix. The water-powder ratio of 0.40 has been used as a standard thickness of the mix (Curve 3). If 0.5 c.c. less water is used to 50 Gm. powder (water-powder ratio 0.39), the expansion increases 0.08 per cent (Curve 2). If another 0.5 c.c. water is withdrawn from the mix (water-powder ratio 0.38), the expansion increases 0.23 per cent. If, on the other hand, more water is added to the standard mix, the expansion does not seem to be so readily affected. If 21 c.c. of water is used to 50 Gm. powder instead of 20 c.c. (water-powder ratio 0.42), the expansion decreases only 0.07 per cent (Curve 4).

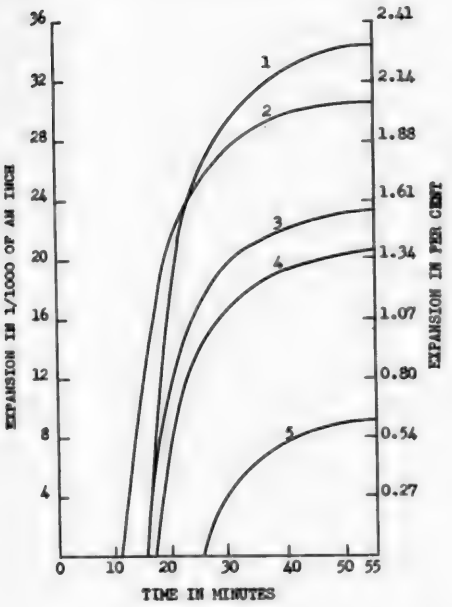

Fig. 9.

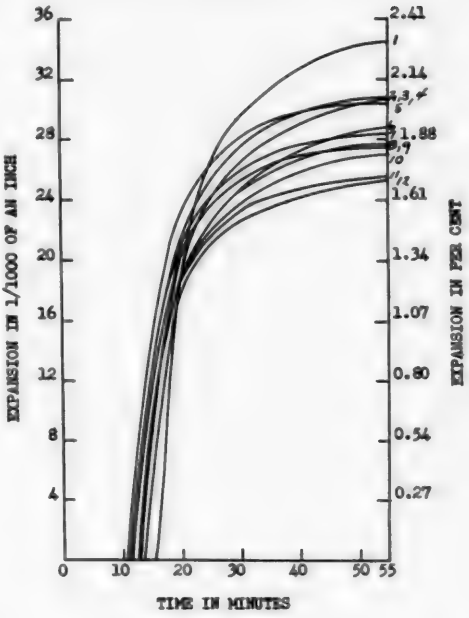

Fig. 10.

Fig. 9.—Beauty Cast Investment. Effect on expansion of time between mixing and immersion.

Fig. 10.—Beauty Cast Investment. Expansion when immersion is made before initial set with variations in water-powder ratio and in temperature of the water bath.

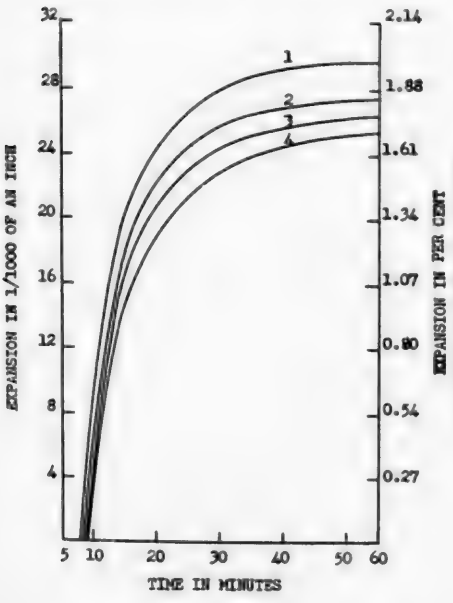

Fig. 11.

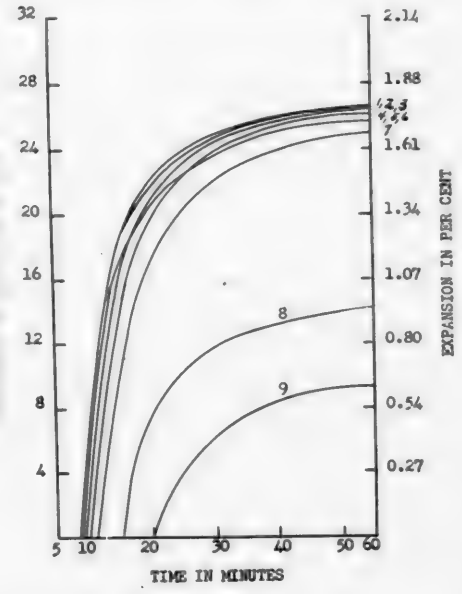

Fig. 12.

Fig. 11.—Cristobalite Investment. Effect on expansion of different water-powder ratios.

Fig. 12.—Cristobalite Investment. Effect on expansion of time between mixing and immersion and of variations of temperature of the water bath.

It is evident from the curves in Fig. 12 and Table IV (B) that the expansion is greatest when the immersion in the water bath is made between 2 and 12 minutes after mixing. Between these times, the results are uniform, which is a good quality, as in some types of patterns the time necessary for investing is longer than in others. The increased temperature of the bath seems to have a slight effect of increasing the hygroscopic expansion (Curves 1 and 2). If immersion is made after initial set, the hygroscopic expansion decreases with increased time between mixing and immersion (Curves 8 and 9).

TABLE IV

CRISTOBALITE INVESTMENT

CURVE	WATER-POWDER RATIO	TEMP. OF WATER BATH (° C.)	INITIAL SET (MIN.)	TIME OF IMMERSION (MIN.)	MAXIMUM EXPANSION (%)
A					
1	0.38	37	13	immediate	1.99
2	0.39	37	14	immediate	1.84
3	0.40	37	15	immediate	1.76
4	0.42	37	16	immediate	1.69
B					
1	0.40	50	15	immediate	1.77
2	0.40	50	15	immediate	1.77
3	0.40	37	15	immediate	1.76
4	0.40	37	15	immediate	1.73
5	0.40	37	15	8	1.72
6	0.40	37	15	12	1.71
7	0.40	37	15	10	1.67
8	0.40	37	15	15	0.95
9	0.40	37	15	20	0.63

CONCLUSIONS

1. Hygroscopic expansion decreases with the increased time between mixing and immersion when immersion is made after initial set.

2. When the investment is immersed after initial set, the temperature of the water bath affects the expansion slightly. Higher temperature of the bath gives increased expansion.

3. When the investment is immersed before initial set, the greatest expansion is obtained and the results are most uniform.

4. There is a slight increase of the expansion when two asbestos linings are used instead of one.

5. Hygroscopic expansion of some investments is not great enough for full compensation of the gold shrinkage.

6. The expansion of some investments is more readily modified than others by differences in water-powder ratio and temperature of the water bath.

7. The casting investment designed for hygroscopic expansion technic gives more uniform results than those primarily designed for thermal expansion.

8. The method of choice in hygroscopic expansion technic is to immerse the specimen 2 to 12 minutes after mixing and keep the specimen in the water at 37° C for 50 minutes.

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CLINICAL OBSERVATIONS ON A CONTRACTING AMALGAM ALLOY

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RESEARCH has clearly indicated that the cause of amalgam failures, other than those due to improper cavity preparation, can no longer be attributed to the use of an inferior alloy, but rather to the improper use of a good alloy.[1] Specifications established by the American Dental Association have assured the dentist alloys of good physical properties, but all of the properties are seriously altered by certain manipulative variables. The dimensional change, in particular, fluctuates greatly with trituration time, moisture contamination, and packing pressure. A slight undertrituration produces a setting expansion above the upper limit of 13 microns per centimeter, which is allowed by the specification, while a slightly overtriturated amalgam will not expand up to the lower limit of 3 microns. The use of mechanical amalgamators and pneumatic or automatic condensers also reduces the expansion and generally produces an actual contraction of several microns.[2] Undoubtedly, many amalgam restorations are being placed daily which do not expand between 3 and 13 microns per centimeter. Some investigators, however, have felt that slight contraction in the amalgam restoration might not be clinically significant and that the increased strength and better handling characteristics associated with a slightly overtriturated amalgam would actually be advantageous.[3]

In a previous investigation, 34 amalgam restorations were placed in the permanent teeth of child patients by a technic which produced a setting contraction of 2 to 4 microns.[4] After a 3-year observation period, there was no definite clinical evidence of contraction and the fillings handled in this manner proved to be slightly superior in terms of roughness and tarnish. The results of this study indicated that a slight contraction or expansion is not clinically significant. Naturally, the tremendous expansion of several hundred microns caused from moisture contamination is quite readily evidenced in amalgam fillings, but the slight deviation induced by a modification of the mixing time might not be clinically important. It should be pointed out that although the dimensional change is generally expressed in terms of microns per centimeter, the actual change across a typical restoration is much less since the width is generally less than 0.5 cm.

It was felt that these previous results[4] should be exaggerated by the use of a special composition alloy. Since most present popular alloys will not contract more than 10 microns, even with prolonged trituration, a 32 per cent tin alloy was compounded by Baker and Company for use in this study.* The technic for mixing and condensing this alloy was standardized in the laboratory

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*The exact composition of this alloy was: Silver, 62.25%; Copper, 4.14%; Tin, 32.25%; Zinc, 1.36%.

so that an average contraction of 31 ± 3 microns per centimeter could always be obtained. This technic, as nearly as humanly possible, was then employed for placing the fillings in the mouth. With careful standardization of all the variables it was anticipated that this procedure would produce fillings with the same properties as were obtained in the laboratory tests. Thus, if no clinical manifestations of contraction were evidenced in these restorations which contract 31 microns, then it would seem logical to predict that the contraction of 3 to 5 microns induced by a slight overtrituration of an accepted alloy or by the use of certain mechanical devices would not be clinically significant.

PROCEDURE

Sixty restorations were placed in the permanent teeth of 32 child patients who were between the ages of 8 and 14 years. Since the main criterion for detecting contraction would be in the form of recurrent caries at the margins, it seemed logical to select an age group having as high a caries index as possible. Most of these child patients were especially suitable for this investigation since their mouth hygiene was poor. Small discrepancies in these fillings would develop more readily into flagrant flaws than in mouths showing good care.

The rules for Black's cavity preparation were consistently followed throughout with restorations being placed in Class I, II, and V types of cavities.[5] This special alloy did contain over 1 per cent zinc, so precautions were taken to avoid moisture contamination and the resulting expansion which would obliterate any setting contraction.[6] The rubber dam was used almost routinely, but in the few cases where it was not feasible, cotton rolls were employed. The trituration was done by means of a Crescent mechanical amalgamator since it has been shown to give slightly more uniform mixes than a hand technic.[2] The restorations were condensed with hand instruments following accepted procedures. In order to assure good margins, the carving of the restoration was delayed for 15 minutes because of the slow setting time of this alloy. This corresponded with the fiducial reading used in the laboratory interferometer tests. All restorations were checked to make certain that the margins were sealed and none of the fillings were polished.

RESULTS

Of the original 60 restorations, 54 have been examined. Thirty-four of these were examined through a period of at least 3 years and 20 for shorter periods of time. All clinical examinations were made with the aid of a mouth mirror and sharp explorer. Most of the restorations were also checked by other members of the faculty, who were not aware of the nature of this problem.

In no restoration was there any observable clinical evidence of contraction which would manifest itself in open margins and/or recurrence of caries. As the sharp explorer was drawn from the tooth structure across the margin of the restoration, there was no catch of the point. Representative fillings, after 3 years, are seen in Figs. 1, 2, and 3. Any slight leak in the margins of these restorations would be expected to have been evidenced as recurrent caries in at least some of the teeth. These particular photographs show mouths in which

the oral hygiene was above the average. On many of the patients, the adjoining teeth became almost completely destroyed by caries with no visible change on the margins of the experimental filling.

It must not be construed that such an alloy as this should be employed. The strength of this alloy is low, its setting time is much too long, and its handling characteristics are inferior. Certainly an accepted alloy which meets the

Fig. 1.

Fig. 2.

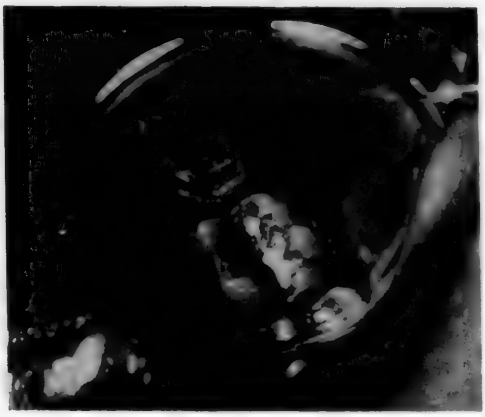

Fig. 3.

Figs. 1 to 3.—Contracting amalgam alloy restorations at the end of the 3-year observation period.

American Dental Association specifications should be employed. Neither does this research indicate that any principle in the amalgam procedure can be disregarded. Success of the clinical restoration is dependent upon control of all variables and any deviation from this standardized technic can only lead to failure. However, the results of this investigation do indicate that the use of a sound technic employing these recommended alloys, but which may produce a slight contraction of the restoration, will not result in clinically significant

defects. The observations which have been made on the contracting restorations are the result of a 3-year study and, while it is difficult to foresee any further changes in the fillings, they will remain under observation as long as the experimental group can be kept intact.

Upon examination of a large number of amalgam restorations, one can readily find numerous defective fillings, some of which when casually observed appear to have contracted or pulled away from the margins of the cavity. These are often spoken of as "ditched fillings" and probably result from insufficient width of the preparation, improper extension, and poor condensation rather than contraction of the alloy.[1, 7]

CONCLUSIONS

Sixty contracting amalgam restorations were placed in the mouths of child patients. 34 observed over a 3-year period and 20 for shorter periods for the purpose of determining the clinical significance of contraction.

There was no clinical evidence of open margins or recurrent caries in the restorations which contracted up to 31 ± 3 microns.

It is apparent from this study that the contraction which may result from slight overtrituration of an alloy or the careful use of mechanical amalgamator or automatic condenser will not be clinically observable. Results of this investigation should not be construed as a recommendation for use of an unaccepted amalgam alloy or deviation from a careful technic.

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LOW TEMPERATURE POLYMERIZATION OF ACRYLIC RESINS

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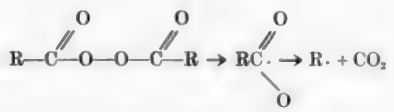
DENTISTRY finally has been presented with resins which will polymerize at room and body temperatures to a hard and esthetic mass. The so-called "self-curing" resins are being received with open arms and not in all quarters with the reservations that some past experience might dictate.

Although we do not feel that we are reactionary, we are of the opinion that some of the properties and characteristics of the resin should be considered and evaluated in terms of established requirements. The "self-curing" resins, if they are to find dental application for all restorations, should fulfill the requirements which have been accepted as minimum standards for material polymerized in the normal manner. In some aspects, there appears to be reasonable doubts as to whether or not these products will prove to be satisfactory for general use.

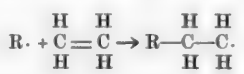
Some attention in the discussion should be focused upon (a) color stability; (b) mechanical properties; (c) degree of polymerization, and (d) properties generally referred to as accuracy of reproduction and dimensional stability. Some phases of these properties will be discussed with the hope that the results may be supplemented, complemented, or refuted, and that we all may have a better appreciation of "self-curing" resins.

In considering the polymerization reaction of "self-curing" resins, the three stages of the polymerization reaction have an increased importance because in this instance each stage, initiation, propagation, and termination, must be considered almost as a separate entity in order to understand and appreciate the manner in which the phenomena take place and to attempt to explain some of the observations.

The initiation stage of the polymerization reaction is actually a combination of two phases. The first phase of the initiation stage is the decomposition of the peroxide to form free radicals



The free radicals having been formed, the second phase of the initiation stage takes place, the attachment of the peroxide-free radical to the monomer molecule with the accompanying opening of the double bond and the shift of the open valence from the peroxide to the monomer molecule



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[Let us represent $R-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}\cdot$ by $RM\cdot$ and $\underset{H}{\overset{H}{C}}=\underset{H}{\overset{H}{C}}$ by M]

Propagation follows immediately in this fashion:

$$RM\cdot + M \rightarrow RM_2\cdot$$

and so on to $RM_M\cdot$

After having propagated to stage $RM_M\cdot$ the chain finally terminated in one of the following manners:

(a) By linkage of two activated chains $RM_M\cdot + RM_M\cdot \rightarrow RM_M{:}M_MR$ or by

(b) Disproporationation $R'-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}\cdot + R''-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}\cdot \rightarrow R'-\underset{H}{C}=\underset{H}{C} + R''-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{CH}}$

In the above reactions, once the polymerization procedure has been initiated each individual chain is formed in a short period of time.

The rate at which a mass of material will polymerize to a substantially high degree is primarily a function of the frequency of occurrence of the initiating stage. This frequency, in turn, will be a function of the rate of decomposition of the catalyst. The problem of causing the monomer to polymerize at room temperature is dependent upon obtaining some compound which will quickly decompose a suitable catalyst to form a free radical capable of initiating polymerization. A suitable and generally used catalyst is benzoyl peroxide. Benzoyl peroxide also has the advantage of having had considerable studies reported in the literature. Bartlett and Nozaki[1] have shown that when, at 80° C., benzoyl peroxide is dissolved in various classes of solvents the rates of decomposition are approximately in the following order: least decomposition in highly halogenated solvents, increasing through aromatics, aliphatics, esters, alcohols, monohydrics phenols to amines. At the temperature studied the benzoyl peroxide was found to decompose at an explosive rate in the amines.

A rough check was made by comparing the rate of decomposition of benzoyl peroxide in several of the compounds studied by Bartlett and Nozaki to ascertain whether the rates of decomposition would fall in approximately the same order at lower temperatures. This evaluation was based upon comparison of curing characteristics when using various solvents to decompose the benzoyl peroxide. Our qualitative results indicated substantially the same order. The most promising agents to aid in the decomposition appear to be the amines.

A survey of a great many amines revealed that, in general, the aromatic amines are greatly superior to the aliphatic amines with respect to their ability to accelerate polymerization at room temperature. It has been found that, when using aliphatic amines, only tertiary amines will satisfactorily decompose the peroxide catalyst at low temperatures. The tertiary amines are also the most effective type of aromatic amines for this purpose. Since the amines discolor upon oxidation, it is to be anticipated that any system which is caused to polymerize by the presence of an amine and a peroxide which is being decomposed, will discolor and, furthermore, the discoloration should be expected to

increase with an increased concentration of the amine and/or the peroxide. The results obtained by varying the concentrations of the amine and the peroxide, independent of each other, roughly bear out this prediction. The type of oxidation of the amine and the resultant discoloration is different when illuminated by ultraviolet or infrared light.

Due to their greater stability the aromatic amines, in general, cause the polymerization to proceed more quickly than when using the aliphatics. As might be anticipated, less discoloration occurs when using the aliphatic amines. Since the discoloration is assumed to be an oxidation reaction, one remedy which immediately suggests itself is the use of active antioxidants, such as hydroquinone, sorbose, etc. This remedy has not proved to be satisfactory. A more reasonable solution to the color problem would be to employ a compound capable of initiating polymerization but which would not, as a secondary reaction, discolor.

The double bond of the monomer must be attacked so as to form a free radical if polymerization is to be initiated. One means of accomplishing this reaction has been used effectively for many years in the vulcanization of rubber. However, since we wish to carry this procedure out at normal ambient temperatures, we must have a sulfur compound which will free sulfur readily when in contact with other compound, e.g., P-toluenesulfinic acid, when dissolved in methyl methacrylate monomer, somehow liberates suitable sulfur to initiate the polymerization reaction. The products of this reaction, when subjected to the usual color stability tests will not discolor. A specimen of "self-curing" acrylic, when polymerized using this compound as an activating agent, will show no discoloration after the normal 24 hours under ultraviolet light.

TRANSVERSE TESTS

The materials employed in the transverse test are activated, using benzoyl peroxide and dimethyl-M-toluidine and dimethyl aniline, represented by T and A, respectively, and the P-toluenesulfinic acid (without the peroxide) identified as S. The transverse test employed the apparatus specified for this test by the A.D.A. Specification No. 12. The test itself was arbitrary insomuch as an initial load of 1200 Gm. was used. The specimens were not ruptured and curves were plotted with the deflection as a function of the load, using 500 Gm. increments. From these curves the results presented in Table I were obtained Columns I, II, and III give the results obtained when the "self-curing" resins were processed at room temperature (approximately 75° F.).

TABLE I

	I	II	III	IV	V
LOAD (KG.)	T DEFLECTION (MM.)	S DEFLECTION (MM.)	A DEFLECTION (MM.)	S DEFLECTION (MM.)	A DEFLECTION (MM.)
2.2	0.75	1.10	0.95	0.85	0.85
3.2	2.00	2.30	2.05	1.75	1.95
4.2	3.50	4.5E	3.4E	2.85	3.3E
5.2	5.6E	6.6E	6.0E	4.30	3.3E
				8.0E	

E = Erratic results.

Material A will polymerize in approximately 20 minutes from time of mixing. Material T will polymerize in approximately 30 minutes from time of mixing. Material S will polymerize in approximately 2 hours from time of mixing.

None of the specimens tested would comply with the A.D.A. Specification No. 12. That these mechanical properties are inferior to those which are observed for normally cured acrylic resins is to be expected since, in these so-called "self-curing" polymerizations, more than the normal number of free radicals are formed per unit time, which would lead one to anticipate the formation of shorter chains. This property will also show lower values due to the presence of free monomer.

In order to investigate the validity of the assumption of shorter chains, number average molecular weight determinations were made, using the method reported by Caul.[2] Materials A, T, and S were found to have number average molecular weights of 3,000, 3,700, and 3,500, respectively. The number average molecular weight may be low, due to short chain length or the presence of free monomer. In order to eliminate free monomer from the specimens after curing they were subjected to boiling water for one-half hour. This is assumed to eliminate the free monomer either by polymerization or evaporation. The molecular weight incerased for Material A from 3,000 to 8,500, for Material T from 3,700 to 19,700, and for Material S from 3,500 to 27,000. The effects of this treatment upon the physical properties is shown by the transverse tests in Columns IV and V (Table I). In the case of Material A where there was not a very great increase of number average molecular weight upon boiling, the effect upon physical properties was not so pronounced as for Material S, which showed a much greater effect.

The free monomer which was originally present after the room temperature curing for the three specimens was found to be as follows: For materials A, T, and S, respectively, 2.18 per cent, 2.20 per cent, and 2.50 per cent.

In order that these values may be compared with something more familiar, two denture materials were processed by curing at 160° F. for 4 hours and compared with the same materials cured by heating for 3 hours at 160° F., followed by 1 hour boiling. For the low temperature the number average molecular weights were found to be 14,000 and 13,000 and the same materials cured by the supplementary 1 hour boiling were found to have number average molecular weights of 47,000 and 49,000. Upon the above basis, the free monomer calculated to be present after the low temperature cure was calculated[2] to be 0.50 per cent and 0.57 per cent.

Another rather interesting comparison can be made from observations of room temperature polymerized methyl methacrylate in one case activated as compared with the results of methyl methacrylate unactivated. A sample of methyl methacrylate monomer which had polymerized in the bottle, due to standing too many years on the dealer's shelf, was found to have a number average molecular weight of 5,000 and after boiling the specimen the molecular weight was still only 5,000. A comparable sample was made from monomer to which was added benzoyl peroxide and dimethyl aniline and no polymer. Al-

though this specimen was comparatively small (approximately 1 inch in diameter and ¼ inch thick) the heat of polymerization was sufficient to cause the specimen to boil during polymerization reaction. Number average molecular weights of this specimen as cured in an open container and subsequently placed in boiling water were both 3,000.

A further check upon the amount of free monomer present in the polymer specimen was deemed advisable, due to the likelihood that the boiling procedure might not have eliminated substantially, all of the free monomer. Accordingly, the specimens were dissolved in chloroform and the polymerized portion of the specimen was precipitated from this solution by addition of methyl alcohol. The number average molecular weight of the precipitate was then determined.

Table II is a tabulation of the number average molecular weights shown by the original specimens after boiling and after precipitation.

TABLE II

MATERIAL	ORIGINAL SPECIMEN	AFTER BOILING	AFTER PRECIPITATION
A	3,000	8,500	32,500
T	3,700	19,700	75,000
S	3,500	27,000	96,000
I	3,000	3,000	21,200
II	5,000	5,000	74,000
III	38,000	----	275,000

Material I represents unactivated monomer polymerized on dealer's shelf.
Material II represents polymerized activated monomer.
Material III represents methyl methacrylate molding powders widely used in dentistry.
A solution of the monomer which had polymerized in the bottle after years of storage possessed an abnormally high viscosity for the low molecular weight.

From the results as shown in Table II it is obvious that the free monomer has not been completely eliminated by boiling the specimen. The free monomer calculated to be present in each of the above specimens is not only different, but not necessarily in the same relative order if the calculation be based upon the original number average molecular weight as compared with after boiling or the values for the precipitated polymer.

The free monomer calculated on each basis is tabulated in Table III.

The dimensional stability has been evaluated by determining the linear dimensional change on one surface of a specimen, 77 mm. × 35 mm. × 4 mm.

TABLE III

MATERIAL	PER CENT MONOMER ORIGINAL-BOILING	PER CENT MONOMER ORIGINAL-PRECIPITATION
A	2.18	3.02
T	2.20	2.58
S	2.50	2.80
I	0	2.89
II	0	1.88
III	–	0.19

Markers of 22 karat plate were imbedded in stone molds. The molds were tinfoiled, leaving windows over the markers in such fashion that the tinfoil overlapped the edge of the marker approximately 2 mm. After the stone molds had set 24 hours the distance between reference lines on the markers were measured.

Specimens were cured at room temperature and after removal from the mold, stored in a room temperature desiccator (sulfuric acid used as descant). Due to the difficulty of making accurate measurements by the procedure adopted, very early observations were of no value. To determine curing shrinkage, measurements were made at 2- or 3-day intervals, commencing when the specimen was 2 days old. Curing shrinkage was considered to be the value obtained when the specimen failed to exhibit further shrinkage in the period of 1 week. After the specimens failed to show further shrinkage they were stored in water at room temperature and dimensional changes were observed at 2-day intervals until no further changes were observed over a period of 1 week. The total changes observed appear in Table IV.

TABLE IV

MATERIAL	CURING SHRINKAGE (PER CENT)	EXPANSION IN WATER (PER CENT)
A	0.43	0.27
T	0.42	0.27
S	0.55	0.50
Denture base resin	0.39	0.10

The denture base resin is included in Table IV as a basis for evaluation of the results obtained for the "self-curing" resins since it is well established that the idiosyncrasies of the mold employed play a significant role in the values obtained.*

Some indication of stability of shape can be inferred from recovery after loading in the transverse test. A few specimens were examined for this feature, and as long as the total load did not exceed 3.5 kg., the recovery was not much less than would be expected of normally cured materials, e.g., the recovery from a deflection of 4.8 mm. to 1.3 mm.

CONCLUSIONS

1. It seems reasonably safe to state that it is possible to make "self-curing" resins with perfectly satisfactory color stability.

2. Results so far indicate that the mechanical properties are not equal to the properties which have been thus far required of resins for prosthetic purposes. If these materials do prove to be satisfactory, it would suggest that our standards for base materials have been unnecessarily high. Mechanical properties and degree of polymerization appear to be comparable with those that would be obtained if a standard base material were considerably undercured.

3. The degree of polymerization appears to be lower than is desirable.

4. If the dimensional stability be considered a function of dimensional changes associated with curing shrinkage and watersorption, the above observations must lead to the conclusion that if the resins be used as a repair material, they should approximately equal the base resins, provided they are kept wet, since the net change curing shrinkage-watersorption is about that associated with the denture base resins.

*The dimensional change observations presented at the time the paper was read were based upon short time studies, which led to tentative conclusions which are refuted by the larger term observations appearing in Table IV.

As a filling material, so far as dimensional changes are concerned, it is equal to an amalgam which would not be in compliance with the A.D.A. Specification No. 1, by virtue of its 15 micron/cm. contraction. However, the dimensional changes may prove to be somewhat different if the measurements be made on a specimen immersed in water at the earliest moment after the specimen has reached a substantial degree of cure. At least as far as filling material is concerned, such measurements would be more significant.

For small deflections the "self-curing" resins are probably approximately equal to normally processed denture base materials. However, they will not withstand, without significant set, as great a load as will normally processed denture base materials.

"Self-curing" acrylics probably will find many excellent uses in dentistry. On the other hand, applications such as filling materials, denture base materials, or denture base repair materials should be approached with some reservation, unless these applications are employed with the idea that they may be of a temporary nature and with only a hope that they will be permanent.

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THERMAL CONDUCTIVITY OF DENTIN

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KNOWLEDGE of the thermal conductivity of dentin is valuable because there are several instances in which heat flows through dental tissues. During cavity preparation, frictional heat is created by the bur in contact with tooth structure. The number of calories produced is dependent upon such factors as the nature of the cutting instruments, the speed and pressure which is used, the surface area involved, and the time of contact with the surface. The presence or absence of moisture, the temperature of the surrounding air, and the distance of the pulp tissue from the cutting instrument influence the quantity of heat. Many materials now used in restorative dentistry create heat. These include oxyphosphate cements, zinc silicates, silicate cements, and especially the new resinous filling materials. The metals used for restoring teeth conduct heat to or from the oral cavity. Whether or not heat on the external surface of dentin will affect the pulp depends on several factors, including the thermal conductivity of the intervening dentin tissue.

The object of this investigation was to determine the thermal conductivity of dentin. The thermal conductivity would affect conclusions concerning the permissible amount of heat that can safely be applied to dentin. It is also important for developing or modifying methods for cavity preparation, for giving specifications for heat conductivity of filling materials, and for amounts of heat permissible during their manipulation.

A review of literature reveals no reference to thermal conductivity of dentin. This is not surprising since the standard methods used for determining heat conductivity use samples of test material larger than could be obtained from a human tooth. The largest sample of dentin obtainable with consistancy was a piece 6.35 mm. in diameter and 1 to 2 mm. in thickness. Such pieces could be obtained from first and second molars.

EXPERIMENTAL PROCEDURE

A molar tooth was mounted on stone blocks and the occlusal surface of the tooth was ground away at right angles to the long axis of tooth with a large stone under a stream of water. After all of the occlusal enamel had been removed, the tooth presented a flat surface of dentin surrounded by a thin layer of enamel. A cutting tool whose inside diameter was 6.35 mm. was used to drill 4 mm. deep into the tooth. The cylinder of dentin produced was sectioned parallel to the flat surface by means of jewelers' saws producing one or more discs of dentin 6.3 mm. in diameter and 1½ mm. in thickness. The discs were measured with a micrometer and then hand polished on a fine stone under

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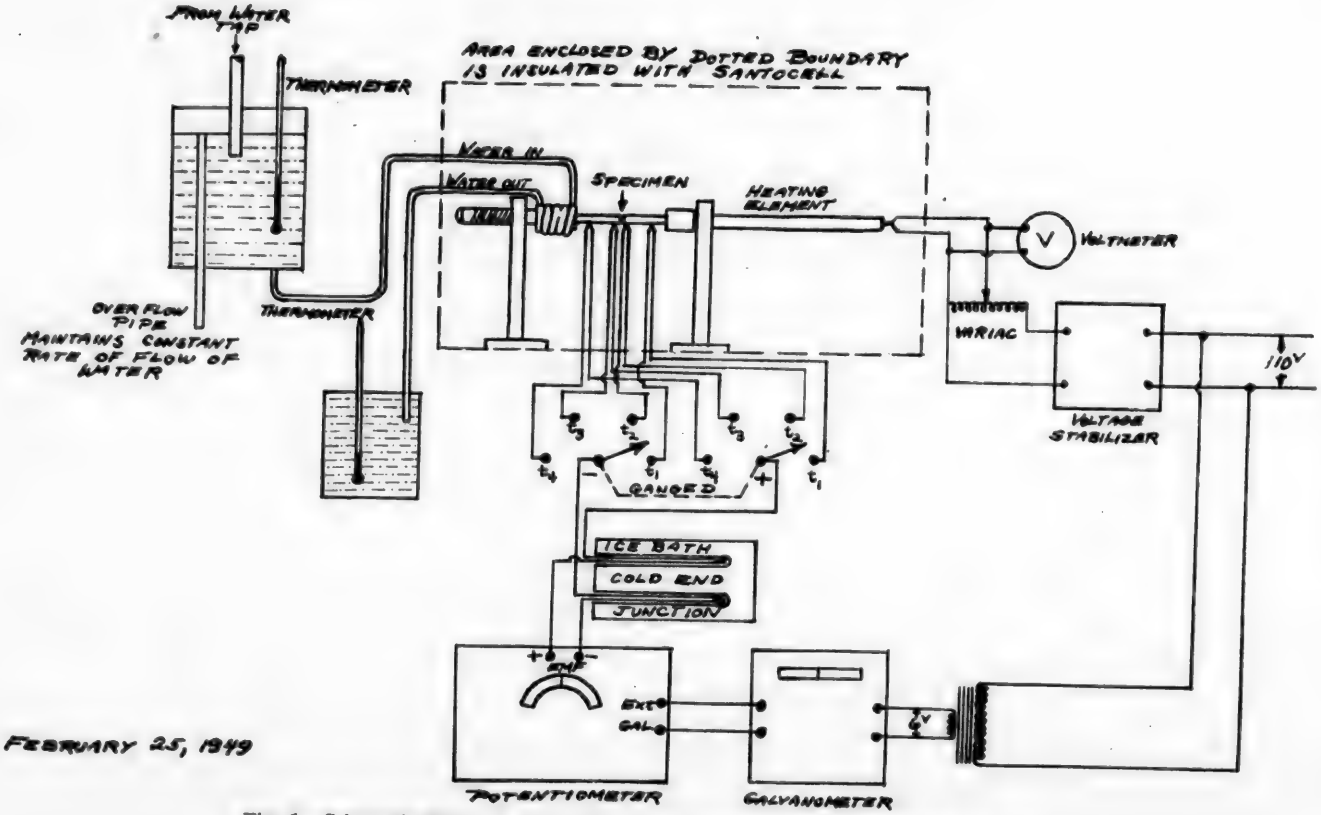

Fig. 1.—Schematic diagram of apparatus to measure thermal conductivity of dentin.

water. The surfaces were then checked against glass for flatness and parallelism. Finally a microscopic examination was made for pits and scratches.

An apparatus was constructed for measurement of heat conductivity of these small sample discs. A diagram of the apparatus can be seen in Fig. 1. The area indicated on this figure by a dotted boundary contains the essential position of the apparatus. It consists of two pure copper rods of the same diameter as the dentin specimen mounted on two retainers with spring tension in order to provide good alignment and contact with the dentin specimen. These heat conducting rods are termed "thermodes." One thermode serves for introducing and the other for removing the heat. Four iron-constantan thermocouples were placed at 1 cm. intervals along the thermodes and in immediate contact with each surface of the sample. The heat input thermode was heated electrically and controlled with a variac. Line voltage was maintained with a G.E. voltage stabilizer. The heat flowed through the input thermode, through the sample, and into the output thermode where it was carried away by a constant flow of water through a cooling coil soldered to the output thermode. Temperature readings from the four thermocouples permitted calculation of the amount of heat flowing through the thermodes and sample. The portion of apparatus outlined in the dotted rectangle in Fig. 1 was buried in silica aerogel, an excellent insulating material. The whole apparatus was permitted to stabilize itself by taking readings occasionally until the temperature readings were constant for 30 minutes. This usually required a period ranging from 24 to 172 hours. At this time, five readings were taken every 5 minutes in order to ascertain that equilibrium was reached.

The apparatus was standardized against samples of Pyrex of known thermal conductivity. These had the same diameter and thickness as the dentin samples to be used.

If a temperature gradient in excess of 100° C. was used, the dentin became discolored and charred. For this reason, the investigation was confined to gradients of 40°, 70°, and 90° C.

After many preliminary tests, the dentin samples were used for thermal conductivity determinations. Readings were first taken at a differential of 40° C., then the heat was increased to cause a 75° C. differential, and finally to induce a 90° C. differential. Duplicate readings were found to check well for seven samples and the initial readings on the seven are reported.

RESULTS

The results obtained from the seven samples are arranged in Table I. K is the value of thermal conductivity. The reading in milliamperes from each of the thermocouples was converted to degrees centigrade from a table supplied by the manufacturer.* The quantity of heat flowing through the sample was:

$$Q = \frac{K \triangle T A}{L}$$

where Q = quantity of heat in calories
K = thermal conductivity of copper
$\triangle$ T = temperature differential, ° C.
A = cross sectional area of thermodes
L = distance between the thermocouples on thermodes (1 cm.)

*Standard Conversion Tables, Leeds and Northrup Company, Philadelphia, Pa.

The thermal conductivity of dentin was obtained from the formula:

$$K = \frac{Q\ l}{\triangle\ t\ a}$$

when Q is obtained from above
l = thickness of dentin
△ t = temperature differential across dentin
a = cross section of sample

By means of these formulas, the average values for thermal conductivity of seven dentin sections ranged from 2.10×10^{-3} to 2.49×10^{-3} with the average mean value for all of them of 2.29×10^{-3}. In other words, a cube of dentin 1 cm. on each side will conduct 0.00229 calories per second when the two opposing surfaces of the sample differ in temperature by one degree centigrade.

TABLE I

THERMAL CONDUCTIVITY OF DENTIN SECTIONS
(K = Calories/sec./cm./C.°/cm.²)

SAMPLE NUMBER	K* VALUE	M.T.° C.†	K	M.T.° C.	K	M.T.° C.	AVERAGE K VALUE OF EACH SAMPLE
1	2.57×10^{-3}	41.45	1.90×10^{-3}	75.52	2.18×10^{-3}	87.9	2.22×10^{-3}
2	2.36×10^{-3}	41.6	2.66×10^{-3}	75.8	1.94×10^{-3}	88.1	2.32×10^{-3}
3	2.36×10^{-3}	44.1	2.84×10^{-3}	76.3	2.26×10^{-3}	86.7	2.49×10^{-3}
4	2.37×10^{-3}	44.9	2.37×10^{-3}	78.6	2.14×10^{-3}	86.8	2.29×10^{-3}
5	2.24×10^{-3}	44.7	2.49×10^{-3}	76.5	2.41×10^{-3}	91.0	2.38×10^{-3}
6	2.17×10^{-3}	45.2	1.87×10^{-3}	79.0	2.26×10^{-3}	87.2	2.10×10^{-3}
7	2.58×10^{-3}	39.5	1.87×10^{-3}	73.5	2.31×10^{-3}	86.3	2.25×10^{-3}
Average mean value of K							
	2.38×10^{-3}		2.29×10^{-3}		2.21×10^{-3}		2.29×10^{-3}

*K – Thermal conductivity of dentin.
†M.T.°C. – Mean temperature across specimen.

Table II is a list of the materials that have thermal conductivity comparable to dentin and the thermal conductivity of common filling materials. From the

TABLE II

VALUES OF MATERIALS COMPARABLE TO DENTIN T.C.

MATERIALS	K (× 10^{-3})
Granite	4.5
Gypsum	3.1
Magnesia, Mg. O. Brick	2.7-7.2
Porcelain	2.5-3.9
Glass window	2.5
Ice	2.2-5.0
Dentin Value	2.29
Concrete, stone	2.2
Silica brick	2.0-3.0
Chalk	2.0
Brick, common red	1.5
Water, 20° C.	1.43
Fire brick	1.1
Air	0.0568
Filling Materials	
Silver	974.0
Gold pure	700.0
Gold alloy CU	164.3
Dentin Value	2.29 .0096
Cement, zinc phosphate	1.14 .0048
Gutta percha	0.48

first part of the table it can be noticed that dentin has a thermal conductivity similar to glass or concrete. In comparison with filling materials, it most resembles zinc phosphate cement.

DISCUSSION

The good agreement of values for dentin at the various temperature gradients suggests that the average is probably near the true value for the thermal conductivity of dentin. A possible source of error is dehydration of the specimen during the test, but there is little difference between the 40° C. differential and the 90° C. differential. If dehydration were an important factor, there should be a larger difference between these averages. Another possible source of error arises from the fact that all of the sections were cross sections with tubular orientation similar, and there can be no prediction of the thermal conductivity with a different tubular orientation.

There are certain limitations in attempting to apply these findings to conditions as they might exist in dental practice. The problem here is whether a certain application of heat will cause a damaging temperature rise in the pulp. In an intact tooth there is constant circulation of tissue fluids within the pulp and within the odontoblastic extensions through the dentinal tubules; this will tend to carry heat away. Heat is also lost by conduction in all directions from its site of generation and only a fraction moves directly toward the pulp. Another limitation of thermal conductivity is the fact that heat radiation has not been considered. A hot dental bur may actually pass more heat to the pulp by radiation than by conduction. Further histological studies of the pulp damage and in vivo investigations of heat conduction are planned in order to provide a better understanding of the tolerance of the tooth to heat trauma.

SUMMARY

An apparatus was constructed for determining thermal conductivity of dentin. The value of thermal conductivity of dentin was found to be 2.29×10^{-3}.

We wish to acknowledge the advice of Professor Gordon B. Wilkes, Heat Measurements Laboratories, Massachusetts Institute of Technology.

THE CITRIC ACID CONTENT OF HUMAN TEETH

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INTRODUCTION

THE citric acid in the soft tissues and body fluids has been quite adequately studied,[1, 2] but less attention seems to have been paid the calcified tissues. Dickens[3, 4] showed that 70 per cent or more of the citric acid in various animals is in the bones and may amount to 1000 mg. per cent of dry, fat-free bone. The citric acid of rats' bones was reported by Class and Smith[5] to be 0.616 per cent and Nicolaysen and Nordbø[6] found 0.50 to 0.66 per cent. Thunberg[7] reported that the bones of birds and fish contained large amounts of citric acid, i.e., 2.63 per cent and 2.67 per cent in the scapula and sternum, respectively, of the black-headed gull, 5.25 per cent in the spine of the herring, and an average of 4.25 per cent in the bones of other species of fish. According to Thunberg,[8, 9, 10] egg shells may contain 0.03 to 0.35 per cent citric acid. Mårtensson[11] found 0.11 to 0.35 per cent citric acid in renal and vesical stones; three salivary concretions contained 0.58, 0.11, and 0.00 per cent citric acid, and two arthroliths from a knee joint contained 0.53 per cent and 0.40 per cent citric acid, but no citric acid was found in gallstones.

Information on the citric acid of bones of domestic animals and man is meager. Thunberg[12] determined the citric acid in the bones of horses, cattle, and man of the thirteenth and sixteenth centuries and found 0.9 to 9.3 per cent, 2.2 to 3.4 per cent, and 0.7 to 1.8 per cent citric acid, respectively. Attempts to correlate the age of the specimens with their citric acid content were accepted with reservation. Thunberg[7] reported the femur, clavicle, rib, and vertebra of man to average 1.62, 1.88, 1.75, and 0.89 per cent citric acid, respectively.

Data on the citric acid of teeth appears to be limited to the work of Thunberg[8] who reported "about 0.5 per cent" in the teeth of cats, cattle, and swine, and to Free[13] who found 540 mg. per cent and 110 mg. per cent in dog's dentin and enamel, respectively. The only report on human teeth appears to be that of Free[13] who found 800 mg. per cent and 90 mg. per cent in the dentin and enamel, respectively.

It appeared of interest, therefore, to add to the data of Free[13] on human teeth and particularly to determine if any relation might exist between the citric acid content and the past dental caries history.

EXPERIMENTAL

The crowns of sound and carious human teeth† were separated into dentin and enamel by the Manly-Hodge technic.[14] Powdered crowns of rats' molars

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†The carious material was removed with a dental bur.

were also carried through the Manly-Hodge procedure to determine if citric acid would be lost in the separation process, aliquot samples being removed at various stages of the procedure and analyzed for citric acid. No loss of citric acid occurred during the separation procedure. Analysis of a large number of dentin and enamel samples of human teeth, after standing for approximately one year, also showed no loss. Thunberg[9] has shown that the values obtained for citric acid in bone substance also did not seem to be influenced by the length of the storage period.

For the determination of citric acid, 25 mg. of dentin and 100 mg. of enamel were dissolved in 15 ml. of 1N HCl with gentle heat, filtered, washed with 5 ml. hot water, and the filtrate and washings received in 50 ml. graduated centrifuge tubes; 0.3 ml. of $18N H_2SO_4$ was added, followed by 0.2 ml. 1M KBr solution and 0.5 ml. saturated $KMnO_4$. The procedure used for saliva[15, 16] was followed from this point, except that the addition of bromine water was found unnecessary. In place of a calibration curve, 2 standards containing 0.090 mg. and 0.150 mg. citric acid in 1N H_2SO_4 were carried through the procedure along with the samples. Constants obtained for these standards were averaged and used for calculation of the results. The constant for each standard was defined as the ratio of the optical density to the concentration, or $k = \frac{D}{0.090}$ and $k = \frac{D}{0.150}$, respectively. A blank consisting of 15 ml. 1N HCl, 9 samples and 2 standards, were carried through the procedure simultaneously and constituted a determination. The average constants for 40 such determinations were 1.98 ± 0.22* and 1.99 ± 0.21* respectively. The concentration of citric acid in mg. per cent in the samples was calculated by the equation $C = \frac{DF}{k}$, where F is the conversion factor which approximated 1,000 in case of the enamel samples and 4,000 in case of the dentin samples.

Sixty-five samples of sound dentin, 129 samples of carious dentin, 39 samples of sound enamel, and 83 samples of carious enamel representing 79 individuals ranging in age from 14 to 64 years comprised the study.

TABLE I

CITRIC ACID CONTENT OF DENTIN OF SOUND AND CARIOUS TEETH

INDIVIDUALS WITH:	SOUND DENTIN		CARIOUS DENTIN		AVERAGE DIFFERENCE	SE△	t	P
	NO. OF INDIVIDUALS	AVERAGE CITRIC ACID CONTENT (MG. %)	NO. OF INDIVIDUALS	AVERAGE CITRIC ACID CONTENT (MG. %)				
Both sound and carious teeth	12	934	12	873	+61.0	30.3	2.01	0.07
Either sound or carious teeth (but not both)	16	854	50	872	-18.0	32.0	0.56	0.58
Combined	—	—	—	—	+13.9	24.7	0.56	0.58

*Mean ± standard deviation.

RESULTS

The first analysis of the data involved the pooling of the results for all sound and carious teeth for each individual. As shown in Table I, 12 individuals had both sound and carious dentin, 16 individuals had only sound dentin, and 50 individuals had only carious dentin.

The data for sound and carious enamel is presented in Table II. Eleven individuals had both sound and carious enamel, 11 individuals had sound enamel only, and 34 individuals had carious enamel only.

TABLE II

CITRIC ACID CONTENT OF ENAMEL OF SOUND AND CARIOUS TEETH

INDIVIDUALS WITH:	SOUND ENAMEL		CARIOUS ENAMEL		AVERAGE DIFFERENCE	SE△	t	P
	NO. OF INDIVIDUALS	AVERAGE CITRIC ACID CONTENT (MG. %)	NO. OF INDIVIDUALS	AVERAGE CITRIC ACID CONTENT (MG. %)				
Both sound and carious teeth	11	100.8	11	100.1	+0.7	9.60	0.066	>0.9
Either sound or carious teeth (but not both)	11	100.2	34	102.0	-1.8	7.61	0.24	0.81
Combined	—	—	—	—	-0.84	5.96	0.15	0.88

The average difference, standard error of the average difference, Fisher's "t" and the "P" values were calculated as follows:

Average Difference = difference between means

Standard error of average difference =

$$\sqrt{\left[\frac{1}{N_s} + \frac{1}{N_c}\right]\left[\frac{\Sigma X_s^2 - \frac{(\Sigma X_s)^2}{N_s} + \Sigma X_c^2 - \frac{(\Sigma X_c)^2}{N_c}}{N_s + N_c - 1}\right]}$$

where N_s = number of individuals with sound teeth

N_c = number of individuals with carious teeth

X_s = individual averages for sound teeth

X_c = individual averages for carious teeth

Combined average difference =

$$\frac{\left[AD_B\right]\left[\frac{1}{(SE\triangle_B)^2}\right] + \left[AD_E\right]\left[\frac{1}{(SE\triangle_E)^2}\right]}{\frac{1}{(SE\triangle_B)^2} + \frac{1}{(SE\triangle_E)^2}}$$

where AD_B = average difference for individuals with both sound and carious teeth

AD_E = average difference for individuals with either sound or carious teeth

$SE\ \triangle_B$ = standard error of average difference for individuals with both sound and carious teeth

$SE\ \triangle_P$ = standard error of average difference for individuals with either sound or carious teeth but not both

$$\text{Combined standard error of average difference} = \sqrt{\frac{1}{\frac{1}{(V_X)^2} + \frac{1}{(V_Y)^2}}}$$

where V_X = standard error of average difference for individuals with both sound and carious teeth

V_Y = standard error of average difference for individuals having either sound or carious teeth but not both

$$\text{Fisher's ``t'' } = \frac{\text{average difference}}{\text{standard error of average difference}}$$

"P" value — Enter Fisher's "t" table in usual manner

In Tables I and II average differences are not significant for either dentin or enamel, assuming a P value of less than 0.05 as significant. There is no evidence, therefore, that differences in the citric acid content of dentin or enamel are associated with caries.

The above statistical analysis is correct if, morphologically, the teeth do not differ with respect to citric acid and if age has no significant effect. To check the first assumption an analysis of variance was undertaken[17] which takes account of effect of tooth differences but not of age differences on citric acid content. This analysis involves computing for each individual with more than one tooth of a given type, a single average for that tooth.

The data are presented in Tables III and IV. Table III consists of fourteen averages while Table IV consists of thirteen averages.

TABLE III

CITRIC ACID CONTENT OF TEETH ACCORDING TO MORPHOLOGY

DENTIN

TOOTH DESIGNATION	SOUND		CARIOUS		ALL	
	CITRIC ACID (MG. %)	NO. OF INDIVIDUALS	CITRIC ACID (MG. %)	NO. OF INDIVIDUALS	CITRIC ACID (MG. %)	NO. OF INDIVIDUALS
Incisors	938	10	864	16	892	26
Canines	909	10	894	8	902	18
1st premolars	934	8	910	9	921	17
2nd premolars	898	5	800	8	838	13
1st molars	815	2	874	10	864	12
2nd molars	903	4	849	23	857	27
3rd molars	885	4	923	19	917	23
Total	912	43	875	93	887	136

TABLE IV

CITRIC ACID CONTENT OF TEETH ACCORDING TO MORPHOLOGY

ENAMEL

TOOTH DESIGNATION	SOUND		CARIOUS		ALL	
	CITRIC ACID (MG. %)	NO. OF INDIVIDUALS	CITRIC ACID (MG. %)	NO. OF INDIVIDUALS	CITRIC ACID (MG. %)	NO. OF INDIVIDUALS
Incisors	125.3	3	90.0	7	100.6	10
Canines	91.8	6	109.6	6	100.7	12
1st premolars	95.2	9	110.5	7	101.9	16
2nd premolars	124.3	5	110.9	4	118.3	9
1st molars	—	—	82.2	8	82.2	8
2nd molars	89.1	3	102.5	17	100.5	20
3rd molars	89.7	4	110.8	18	107.0	22
Total	101.0	30	103.0	67	102.4	97

The analysis of variance according to Snedecor[17] (Section 10.4) for the effect of tooth type on citric acid of dentin and enamel gave an F value of 1.09 and 1.35, respectively, with a probability greater than 0.05 in each case. No significant differences, therefore, are evident in the averages for the citric acid content of the dentin or enamel according to tooth type.

To test the significance of age on citric acid content of dentin and enamel, analysis of covariance according to Snedecor[17] (Section 12.3) was used. F values of 1.08 and 3.52, respectively, were obtained for the effect of age of individuals on citric acid of the dentin and enamel. The probability was greater than 0.05 for both dentin and enamel, with a probability value of 0.06 in the case of enamel. The effect of age on the citric acid content of the dentin or enamel is not significant, although it should be noted that in the case of enamel, a borderline significance may exist.

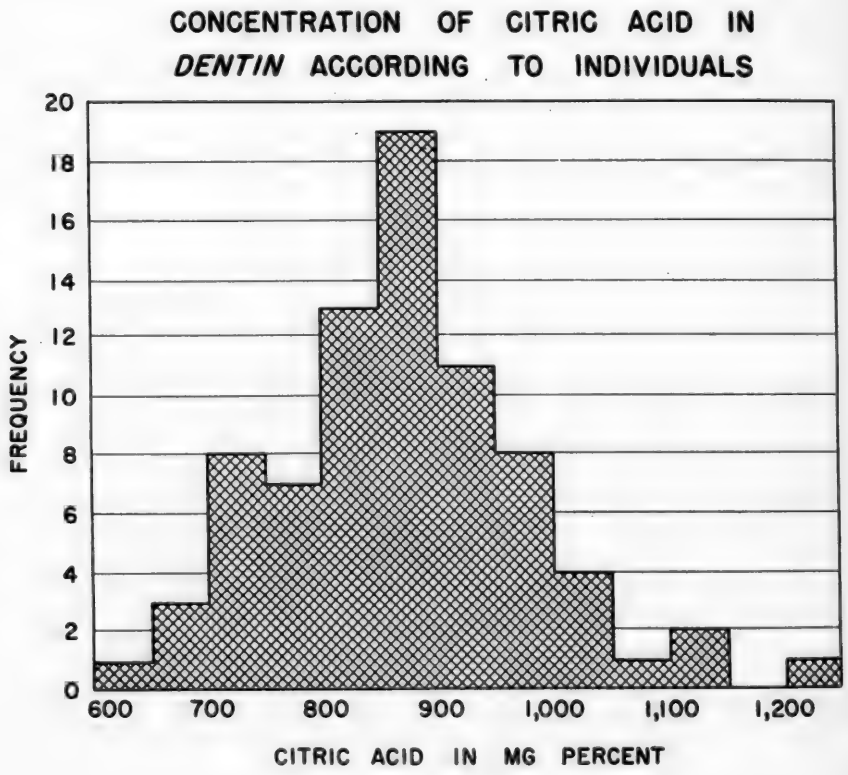

Fig. 1.

Since it appears from these statistical analyses of the data that the citric acid of dentin and enamel is not related to caries, morphology, or age, all the sound and carious teeth were grouped for each individual, and the average taken for the citric acid content of dentin and enamel. The frequency distribution curves of these averages are presented in Figs. 1 and 2. For dentin a

normal distribution curve is obtained and the citric acid content of 875 mg. per cent at the mid-point of the mode agrees well with the calculated mean of 877 mg. per cent. In the case of enamel, however, a curve skewed to the right is obtained with the citric acid content of 85 mg. per cent at the mid-point of the mode as compared with the calculated mean of 99.3 mg. per cent. The following average values were found for dentin and enamel:

SAMPLE	AVERAGE IN MG. PER CENT*
Sound dentin	888.1 ± 103.0
Carious dentin	872.1 ± 105.0
Sound enamel	100.5 ± 22.9
Carious enamel	98.7 ± 26.3

*Mean ± standard deviation.

It will be noted that the range of values obtained for enamel is greater than that for dentin as observed from the distribution curves as well as from the magnitude of the standard deviation.

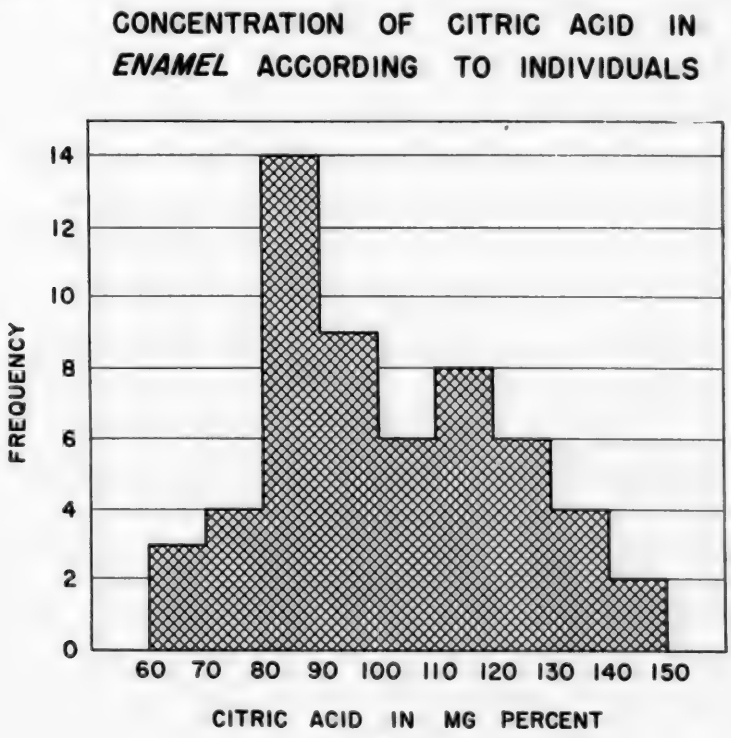

Fig. 2.

DISCUSSION

The role of citric acid in the dental tissues is unknown. Free[13] has shown that the citrate of whole ground teeth is completely insoluble in both petroleum ether and diethyl ether, and that only a small fraction is extracted with water.

Our work indicates also that the citrate of powdered rats' crowns is also insoluble in ethyl alcohol, acetone and bromoform. It appears, therefore, that the citrate is not present as calcium citrate per se or organically bound in a complex soluble in the nonpolar solvents mentioned, but rather is found as an insoluble complex containing both calcium and phosphate. In this connection it is of interest to note that in vitro studies by Kuyper[18] have shown that solutions containing varying amounts of calcium chloride, potassium acid phosphate, and citric acid made alkaline to litmus, gave a precipitate which contained varying amounts of citric acid, and complete precipitation of citric acid was obtained only when the calcium ion was present in excess of the amount necessary to react with the phosphate and citrate. Similarly, the solubility of precipitated calcium phosphate increased with the amount of citrate in the equilibrium mixture.[19] Steinhardt[20] has shown also that citric acid in various concretions and egg shells is accompanied by phosphate and calcium, and that a high citric acid content is associated with a high phosphate and calcium content.

It appears, therefore, that the insolubility of the citrate of dentin and enamel may be explained in part by the high concentration of calcium and phosphate.

SUMMARY

1. Sixty-five samples of sound dentin had an average citric acid content of 888 ± 103 mg. per cent, 129 samples of carious dentin had 872 ± 105 mg. per cent, 39 samples of sound enamel had an average citric acid content of 100.5 ± 22.9 mg. per cent, and 83 samples of carious enamel had 98.7 ± 26.3 mg. per cent.

2. The citric acid content of dentin and enamel of human teeth does not appear to be related to either caries or to tooth type.

We are indebted to Mr. Jerome Cornfield, Statistician, Division of Public Health Methods, Office of the Surgeon General, Public Health Service, for statistical treatment of the data.

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THE LOCATION OF THE GLYCOLYTIC ENZYMES IN SALIVA

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INTRODUCTION

THE PROCESSES of anabolism and growth involve the synthesis by the living cell, from simple sustances of low energy content to complex carbohydrate, protein, and lipid materials which are rich in energy. In general, the energy utilized for synthesis by heterotrophic organisms is obtained either by fermentation (anaerobic oxidation) or respiration (aerobic oxidation) of suitable organic substrates, coupled with the formation of energy rich phosphate bonds.

Most aerobic organisms can break down glucose completely according to the following reactions[1]:

$C_6H_{12}O_6 + O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

$\triangle\ F = 686{,}000$ calories

On the other hand, the anaerobes can only partially break down the glucose molecule, as, e.g., in the lactic acid fermentation:

$C_6H_{12}O_6 \rightarrow 2\ C_3H_6O_3$

$\triangle\ F = 54{,}000$ calories

It has been known for years that fermentation is not produced by the organisms as such, but by enzymes contained in or secreted by them. Fosdick[2] showed that intermediate products in carbohydrate degradation could be isolated from fermentation mixtures of saliva, glucose, and tooth enamel. Although it is now generally accepted that the enzyme systems are derived from the oral microorganisms,[3] it has as yet not been shown whether or not these enzymes are intracellular or extracellular.

The microorganisms are generally removed from liquids by Seitz filtration. Extracellular enzymes, if present in the original sample, would appear in the filtrate, whereas intracellular enzymes would be retained in the residue. It is a well-known fact, however, that numerous substances are adsorbed on to a Seitz filter and thereby removed from solution. In order to eliminate this possibility, one series of samples was filtered through a Seitz filter and another series was filtered through a nonadsorbing Pyrex "UF" fritted glass filter. The filtrates obtained by using either the Seitz or Pyrex filter were found to be free of organisms.

METHODS AND MATERIALS

A 15 ml. sample of paraffin-stimulated saliva was collected in a sterile bottle, thoroughly shaken, and divided into two equal parts. Part 1 was set

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aside as a control for further testing as outlined below. Part 2 was passed through a Seitz or Pyrex "UF" fritted glass funnel, and the filtrate collected in a suitable sterile container. The two solutions were then prepared in the following manner:

Tube 1. Whole unfiltered saliva, 3 ml.; sterile distilled H_2O, 1 ml.

Tube 2. Whole unfiltered saliva, 3 ml.; 10 per cent glucose solution, 1 ml.*

Tube 3. Salivary filtrate, 3 ml.; sterile distilled H_2O, 1 ml.

Tube 4. Salivary filtrate, 3 ml.; sterile 10 per cent glucose solution, 1 ml.*

The tubes were placed in an incubator at 37° C. for twenty-four hours and the following determinations were made on the samples at the beginning and termination of the incubation periods: (1) pH, (2) total titratable acids, using 0.01N NaOH and phenolphthalein as an indicator, and (3) protein breakdown as determined by the Sorenson formol titration.[4]

RESULTS

The results obtained concerning the effect of Seitz filtration of saliva upon its ability to degrade glucose have been arranged in Tables I, II, and III. The effects following the filtration with the Pyrex "UF" fritted glass funnel are in Tables IV, V, and VI.

A. *Results with Seitz Filtration*

Table I demonstrates the effect of Seitz filtration upon the ability of whole saliva and the salivary filtrate to change the pH of a 2.5 per cent concentration of glucose. It was found that the mean pH of the whole saliva of 5 individuals was 7.7 at the start of the experiment and 4.4 at the end of twenty-four hours' incubation at 37° C. This is a decrease in pH of 3.3 units. In contrast to this, Tube 4, which contained the salivary filtrate plus the glucose solution showed even a slight rise in pH, from 7.7 to 8.2.

Table II demonstrates the effect of Seitz filtration upon the ability of whole saliva and the salivary filtrate to produce acid from the 2.5 per cent glucose solution. Tube 2 showed an increase of acids during the twenty-four-hour period, whereas Tube 4 demonstrated a decrease of total titratable acids.

Table III shows the chnges in the Sorenson formol titration. These are not statistically significant and the slight differences evident in the table are attributed to experimental error.

Analysis of these results reveals the following: (1) removal of the bacterial and cellular elements from human saliva by Seitz filtration effectively inhibits the ability of that saliva to produce acid (Table II) or reduce the pH (Table I) of a known concentration of glucose in twenty-four hours; (2) incubation of the salivary filtrate did not reveal an increase in the protein breakdown (Table III), and (3) the results indicate that the enzyme systems involved in the fermentation reaction are intracellular in nature.

B. *Results with the Pyrex "UF" Fritted Glass Funnel*

Tables IV, V, and VI illustrate the results obtained with a Pyrex "UF" fritted glass funnel. Table IV represents the changes in pH; Table V changes in total titratable acids; and Table VI, changes in protein breakdown.

*An aqueous solution of glucose, C.P., was made on a weight per volume basis, i.e., percentage equals Gm. per 100 ml. of solution.

TABLE I

EFFECT OF SEITZ FILTRATION OF SALIVA UPON ITS ABILITY TO DEGRADE GLUCOSE
A. CHANGE IN pH

	TUBE 1			TUBE 2			TUBE 3			TUBE 4		
	WHOLE SALIVA 3 C.C. DIST. H_2O 1 C.C.			WHOLE SALIVA 3 C.C. 10 PER CENT GLUCOSE 1 C.C.			SALIVARY FILTRATE 3 C.C. DIST. H_2O 1 C.C.			SALIVARY FILTRATE 3 C.C. 10 PER CENT GLUCOSE 1 C.C.		
	pH			pH			pH			pH		
CASES	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE
1	7.4	7.8	0.4	7.4	4.4	–3.0	7.5	8.5	1.0	7.5	7.8	0.3
2	7.9	7.4	–0.5	7.9	4.2	–3.7	7.9	8.1	0.2	7.9	8.2	0.3
3	7.5	7.3	–0.2	7.5	4.3	–3.2	7.8	8.1	0.3	7.8	8.2	0.4
4	8.0	7.4	–0.6	8.0	4.8	–3.2	7.8	8.3	0.5	7.8	8.4	0.6
5	8.0	7.3	–0.7	8.0	4.3	–3.7	7.7	8.4	0.7	7.7	8.3	0.6
Mean	7.7	7.4	–0.32	7.7	4.4	–3.36	7.7	8.3	0.54	7.7	8.2	0.44
δ	--	--	0.4	--	--	0.28	--	--	0.28	--	--	0.13

TABLE II

EFFECT OF SEITZ FILTRATION OF SALIVA UPON ITS ABILITY TO DEGRADE GLUCOSE
B. CHANGE IN TOTAL TITRATABLE ACIDS (T.T.A.)*

	TUBE 1			TUBE 2			TUBE 3			TUBE 4		
	WHOLE SALIVA 3 C.C. DIST. H_2O 1 C.C.			WHOLE SALIVA 3 C.C. 10 PER CENT GLUCOSE 1 C.C.			SALIVARY FILTRATE 3 C.C. DIST. H_2O 1 C.C.			SALIVARY FILTRATE 3 C.C. 10 PER CENT GLUCOSE 1 C.C.		
	T.T.A.			T.T.A.			T.T.A.			T.T.A.		
CASES	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE
1	0.2	0.2	0.0	0.2	1.7	1.5	0.2	0.0	–0.2	0.2	0.2	0.0
2	0.0	0.1	0.1	0.0	2.3	2.3	0.0	0.0	0.0	0.0	0.0	0.0
3	0.3	0.4	0.1	0.3	2.6	2.3	0.2	0.0	–0.2	0.2	0.0	–0.2
4	0.0	0.3	0.3	0.0	2.4	2.4	0.2	0.0	–0.2	0.2	0.0	–0.2
5	0.1	0.3	0.2	0.1	3.2	3.1	0.0	0.1	0.1	0.0	0.0	–0.1
Mean	0.1	0.2	0.14	0.1	2.4	2.32	0.1	0.0	0.1	0.1	0.04	0.1
δ	--	--	0.12	--	--	0.53	--	--	0.12	--	--	0.028

*T.T.A. expressed as c.c. 0.01N NaOH required for neutralization; phenolphthalein indicator.

TABLE III

EFFECT OF SEITZ FILTRATION OF SALIVA UPON ITS ABILITY TO DEGRADE GLUCOSE
C. CHANGE IN PROTEIN BREAKDOWN*

CASES	TUBE 1† CHANGE	TUBE 2 CHANGE	TUBE 3 CHANGE	TUBE 4 CHANGE
1	1.7	0.6	–0.2	–0.1
2	0.7	0.5	0.5	0.2
3	0.8	0.5	–0.3	–0.3
4	1.0	0.6	–0.2	–0.4
5	1.1	0.5	–0.3	–0.3
Mean	1.06	0.54	–0.1	–0.18
σ	0.34	0.04	0.3	0.021

*Protein breakdown expressed as c.c. 0.01N NaOH required in Sorenson formol titration.

†Tube 1. Whole Saliva 3 c.c.
Dist. H_2O 1 c.c.
Tube 2. Whole Saliva 3 c.c.
10 per cent Glucose 1 c.c.
Tube 3. Salivary Filtrate 3 c.c.
Dist. H_2O 1 c.c.
Tube 4. Salivary Filtrate 3 c.c.
10 per cent Glucose 1 c.c.

It was found (Table IV) that the mean pH of the 5 samples was 7.6 at the start of the experiment, and 4.1 at the end of twenty-four hours (Tube 2). This is a mean decrease of 2.4 pH units. In contrast to this, there was no change in pH in Tube 4 for the same time period.

Changes in the total titratable acids are shown in Table V. Tube 2 revealed an increase in acids from 0.1 c.c. to 2.0 c.c., an increase of 1.9 c.c., whereas Tube 4 demonstrated no alteration in its acid content.

Table VI shows the changes in protein breakdown as revealed by the Sorenson formol titration. These are not significantly large enough to warrant any conclusions.

An evaluation of these results reveals that the findings are similar to those already obtained by Seitz filtration, with the exception that the problem of adsorption is hereby controlled.

DISCUSSION

The results of the experiments described appear to support the contention that the acids formed by the action of saliva on suitable carbohydrates occur as a result of microbial fermentation. Filtration of the saliva, and subsequent analysis of the action of the filtrate on a known concentration of glucose, reveals a change in its ability to produce acids or alter the pH. The fact that this occurs with both methods of filtration, i.e., Seitz and Pyrex, tends to lend credence to the fact that the enzyme systems involved are intracellular.

It is apparent that future methods designed to exert an inhibitory effect on acid production in the oral cavity must take cognizance of the fact that the enzymes are within the microorganisms.

It would seem, therefore, that measures devised to alter the ability of the saliva to degrade glucose would have to be directed at ways and means of destroying the metabolism of the microorganisms involved in the glycolytic process.

TABLE IV

EFFECT OF FILTRATION WITH PYREX "UF" FRITTED GLASS FUNNEL UPON ABILITY OF SALIVA TO DEGRADE GLUCOSE

A. CHANGE IN pH

	TUBE 1			TUBE 2			TUBE 3			TUBE 4		
	WHOLE SALIVA 3 C.C. DIST. H_2O 1 C.C.			WHOLE SALIVA 3 C.C. 10 PER CENT GLUCOSE 1 C.C.			SALIVARY FILTRATE 3 C.C. DIST. H_2O 1 C.C.			SALIVARY FILTRATE 3 C.C. 10 PER CENT GLUCOSE 1 C.C.		
	pH			pH			pH			pH		
CASES	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE
1	7.5	7.2	−0.3	7.5	4.2	−3.3	8.1	8.5	0.4	8.1	8.2	0.1
2	7.5	7.2	−0.3	7.5	4.2	−3.3	8.1	8.5	0.4	8.1	8.2	0.1
3	7.6	7.0	−0.6	7.6	4.1	−3.5	8.1	8.5	0.4	8.1	8.0	−0.1
4	7.6	7.1	−0.5	7.6	4.0	−3.6	8.1	8.4	0.3	8.1	8.0	−0.1
5	7.6	7.0	−0.6	7.6	4.1	−3.5	8.1	8.5	0.4	8.1	8.1	0.0
Mean	7.56	7.10	−0.46	7.56	4.12	−3.44	8.1	8.5	0.4	8.1	8.1	0.0
δ	--	--	0.13	--	--	0.12	--	--	0.04	--	--	0.09

TABLE V

EFFECT OF FILTRATION WITH PYREX "UF" FRITTED GLASS FUNNEL UPON ABILITY OF SALIVA TO DEGRADE GLUCOSE

B. CHANGE IN TOTAL TITRATABLE ACIDS (T.T.A.)*

	TUBE 1			TUBE 2			TUBE 3			TUBE 4		
	WHOLE SALIVA 3 C.C. DIST. H_2O 1 C.C.			WHOLE SALIVA 3 C.C. 10 PER CENT GLUCOSE 1 C.C.			SALIVARY FILTRATE 3 C.C. DIST. H_2O 1 C.C.			SALIVARY FILTRATE 3 C.C. 10 PER CENT GLUCOSE 1 C.C.		
	T.T.A.			T.T.A.			T.T.A.			T.T.A.		
CASES	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE	START	24 HRS.	CHANGE
1	0.1	0.2	0.1	0.1	2.0	1.9	Alk.	Alk.	0.0	Alk.	Alk.	0.0
2	0.1	0.2	0.1	0.1	2.1	2.0	Alk.	Alk.	0.0	Alk.	Alk.	0.0
3	0.1	0.1	0.0	0.1	1.9	1.8	Alk.	Alk.	0.0	Alk.	Alk.	0.0
4	0.1	0.2	0.1	0.1	2.0	1.9	Alk.	Alk.	0.0	Alk.	Alk.	0.0
5	0.1	0.2	0.1	0.1	2.0	1.9	Alk.	Alk.	0.0	Alk.	Alk.	0.0
Mean	0.1	0.2	0.08	0.1	2.0	1.9	0.0	0.0	0.0	0.0	0.0	0.0
δ	--	--	0.04	--	--	0.006	--	--	0.00	--	--	0.0

*T.T.A. expressed as c.c. 0.01N NaOH required for neutralization; phenolphthalein indicator.

TABLE VI

EFFECT OF FILTRATION WITH PYREX "UF" FRITTED GLASS FUNNEL UPON THE ABILITY OF SALIVA TO DEGRADE GLUCOSE

C. CHANGE IN PROTEIN BREAKDOWN*

CASES	TUBE 1† CHANGE	TUBE 2 CHANGE	TUBE 3 CHANGE	TUBE 4 CHANGE
1	–0.1	0.1	0.0	0.0
2	–0.1	0.1	0.0	–0.1
3	1.0	0.3	0.0	0.0
4	1.1	0.4	0.0	0.0
5	1.1	0.3	0.1	0.0
Mean	0.6	0.2	0.02	–0.02
δ	0.48	0.12	0.04	0.04

*Protein breakdown expressed as c.c. 0.01N NaOH required in Sorenson formol titration.

†Tube 1. Whole Saliva 3 c.c.
Dist. H_2O 1 c.c
Tube 2. Whole Saliva 3 c.c.
10 per cent Glucose 1 c.c.
Tube 3. Salivary Filtrate 3 c.c.
Dist. H_2O 1 c.c.
Tube 4. Salivary Filtrate 3 c.c.
10 per cent Glucose 1 c.c.

SUMMARY AND CONCLUSIONS

An experiment has been described to determine the location of the glycolytic enzymes in the whole saliva. Evidence is presented which indicates that the enzyme systems of the saliva which produce glycolysis are intracellular, i.e., are to be found mainly in the microorganisms of the mouth.

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THE EFFECT OF PARTLY SYNTHETIC DIETS ON THE DENTAL CARIES INCIDENCE IN SYRIAN HAMSTERS

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SEVERAL experimental studies in the past few years have revealed that the Syrian hamster (*Cricetus auratus*) is sufficiently susceptible to tooth decay and sufficiently different from the albino rat in its reaction to various caries-inducing procedures to make it a useful animal for various kinds of dental caries investigations, particularly for certain kinds of studies which have been difficult to make with the rat.

Arnold[1] first demonstrated that molar lesions develop in these animals when they are fed a soft or finely ground carbohydrate diet. The carious process in hamsters was as marked in speed of development and in extent of lesions as the carious destruction encountered in the commonly studied experimental caries of the white rat. Furthermore, lesions in the hamster developed without the occurrence of cusp fractures, which Rosebury, Karshan, and Foley[2] and others described and attributed to the feeding of coarse, hard particle diets such as those developed by Shibata[3] and by Hoppert, Webber, and Canniff[4] and which have been generally accepted as essential to the development of occlusal caries in the white rat. Carious lesions have been encountered in areas of the rat molar which were free of fractures, but these seem to occur less frequently and when found were smaller and slower in their development. The hamster, however, appears to be more susceptible to caries-inducing influences which are not dependent upon preceding fracture of the cusps.

Keyes and Dale, who described the morphological structure of the hamster dentition,[5] confirmed Arnold's findings and gave detailed descriptions of several typical carious lesions developed in the molars of this animal.[6] With the hamster a number of studies have been conducted upon dental caries-producing and caries-inhibiting diets.[7-13] In earlier reports from this laboratory, some of the first work of Keyes and his confreres was confirmed[14] and descriptions were given of the oral bacteria found associated with dental caries in the hamster.[15] These reports were based on data obtained from a portion of the animals used in a series of experiments designed to explore in a preliminary fashion the influence of certain partly synthetic diets upon the incidence of dental caries. This series, involving a total of 894 hamsters† distributed into 18 different dietary groups and including the animals used in the previous studies (except those on commercial rations), provides the material for the present report.

Since many types of bacteria, including those which have been considered important in the etiology of tooth decay, are known to require certain vitamins

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†Of the 894 hamsters which survived the test period, 212 were used in preliminary experiments while the principal study included 682 animals as reported earlier.[14]

or vitamin-like factors for growth and metabolic activity, it was considered possible that a decrease in the amount of tooth decay might result from a deficiency in one or more of these substances in the substrate available as food to bacteria in the oral cavity. Contrary effects may also be present, as has been demonstrated for vitamin K,[16] which by its presence seems to inhibit acid formation by the mixed flora of the saliva. That certain qualitative and quantitative changes in the flora might be expected to occur when certain nutrient substances essential for bacterial metabolism are eliminated from the diet was considered, and such findings have been reported previously.[15] This study, then, concerns itself with the dental caries incidence in hamsters which were fed upon various, partly synthetic diets, each of the orally administered diets being deficient in a simple vitamin which was supplied by parenteral injection.

EXPERIMENTAL METHODS

Stock Colony Diets

The animals used in preliminary experiments were from a stock colony, originally maintained in the laboratory but subsequently kept by an animal supplier, which was fed a commercially prepared food in pellet form.* Each cage was continuously supplied with water and with a supply of food pellets in excess of the amount consumed. The young hamsters removed from the mothers' cages at 21 to 25 days of age, were kept in ⅓ inch mesh wire bottom cages and placed upon the experimental diets. Two lots of the commercially prepared pellets were used as the basal ration of the breeding colony. The first lot contained 20 ppm (parts per million) of fluorine,† according to the manufacturer's analysis. The second lot, stated to contain 7 ppm, was found upon analysis in the laboratory‡ to contain 17 ppm fluorine.

Progeny from this colony, when fed for 100 days on the experimental diets, developed considerably less caries (Table I) than had been found by Arnold who reported 79 per cent of the molars to be carious after 90 to 95 days on fine corn meal diets. It was assumed that previous exposure to fluorides may have been responsible, at least in part, for the unexpectedly low incidence of caries in these experiments, and arrangements were made with the animal supplier to have the colony fed on grains, leafy vegetables, and carrots until a commercially prepared ration low in fluorides could be obtained. On request two manufacturers submitted food samples of supposedly low fluoride content. One of these was found to contain 17 ppm of fluorine, and the other, 1.5 ppm. The latter, in dry meal form, conformed, in preparation and in analysis except for lowered fluoride content, to the standard laboratory animal food prepared by that manufacturer.§ The stock colonies of an additional supplier of hamsters as well as the one previously engaged were fed on this stock ration throughout the remainder of these studies. Weanling hamsters from these stocks, approximately 21 days old when received, were fed for 5 days in the laboratory on the same low-fluoride diet before being placed on experimental diets.

*Rockland Rat Diet, prepared by Arcady Farms Milling Co., Chicago, Ill.

†Fluorine when expressed as parts per million in this paper refers to the fluoride ion.

‡The authors are indebted for the fluoride analyses to Dr. Delbert A. Greenwood, Department of Pharmacology and the American Meat Institute Research Laboratory, University of Chicago.

§Purina Dog Chow Checkers, prepared by Ralson Purina Co., St. Louis, Mo.

TABLE I

DENTAL CARIES IN HAMSTERS IN PRELIMINARY TESTS OF THREE EXPERIMENTAL DIETS

STOCK RATION* FED TO THE BREEDING COLONY		EXPERIMENTAL DIETS FED TO TEST ANIMALS†	NUMBER OF ANIMALS TESTED	NUMBER OF MOLARS EXAMINED	CARIOUS MOLARS DETERMINED BY GROSS OBSERVATION	
RATION LOTS	FLUORINE CONTENT (PPM)				NO.	%
A	20	I	28	336	84	25
B	17	I	5	60	35	58
A	20	II	3	36	4	11
B	17	II	3	36	21	60
A	20	III	3	36	6	17
B	17	III	10	120	67	56

*Commercial Rockland Rat Diet pellets were used. Lot A of this preparation used in the early period of the study (June to Sept., 1944) contained 20 ppm fluorine according to assay figures supplied by the producer, the Arcady Farms Milling Co., Chicago, Ill. Lot B, from the same supplier, was used somewhat later (after Oct., 1944) and was reported to contain 7 ppm, but on analysis at the University was found to have an average fluorine content of 17 ppm.

†These preliminary diets correspond with three of those used later and listed in Table III under the same numbers. All animals were fed 100 days, abiotized, and their molars then examined.

Experimental Diets

Originally, it was planned to prepare the experimental diets, including extraction of casein, in the laboratory but this proved to be both expensive and time consuming. A commercial, vitamin B test diet* was used as the basal ration. Its composition was as follows:

Sucrose	68 per cent
Vitamin-free casein	18 per cent
Vegetable oil	10 per cent
U.S.P. salt mixture No. 2	4 per cent

When it was occasionally necessary to prepare the diet in the laboratory, a vitamin-free casein preparation was obtained from the same commercial supplier.

Vitamins A and D, obtained from another commercial source,† were added to the other vitamin supplements in amounts equal to 2 per cent cod liver oil in the basal diet fed to all the animals and hence were not subjected to test in the study.

The complete vitamin supplement was made up as follows:

Thiamine hydrochloride	50 mg.
Riboflavin	50 mg.
Pyridoxine hydrochloride	50 mg.
Calcium pantothenate	50 mg.
Nicotinic acid	75 mg.
Para-aminobenzoic acid	1 Gm.
Inositol	1 Gm.
Choline chloride	3 Gm.
Biotin	1 ml. (25 μg.)
Alpha-tocopherol (pure)	0.7 ml.
2-methyl naphthoquinone	30 mg.
Vitamin A and D preparation	6 ml.

All vitamins used were pure and in crystalline form; solutions and suspensions of them were kept in the refrigerator.

*Prepared by the S.M.A. Corporation, Chicago, Ill. (now the S.M.A. Division of Wyeth Incorporated, Philadelphia, Pa.).

†Vi-delta, prepared by Lederle Laboratories, Inc. (now Lederle Laboratories Division, American Cyanamid Co., New York, N. Y.)

The effects of the different vitamin B components were tested by elimination. The composition of the 17 different test diets used is shown in Table II. The appropriate supplements were pooled and mixed with 1.0 kg. of vitamin B test diet. This mixture constituted the complete control diet (Diet I). All other diets except Diets VIII and XIV were prepared in the same manner but that one or more vitamins were omitted from the supplement. The missing

TABLE II

COMPOSITION OF EXPERIMENTAL DIETS

DIET NUMBER	BASAL DIET* (WITHOUT VITAMINS)	DELETIONS FROM VITAMIN SUPPLEMENT†	PARENTERAL SUPPLEMENTATION
I	Complete	None	None
II	Complete	Riboflavin	2 mg. riboflavin in saline (steam sterilized)
III	Complete	Calcium pantothenate	2 mg. calcium pantothenate in saline (steam sterilized)
IV	Complete	Thiamine hydrochloride	2 mg. thiamine hydrochloride in saline (Berkefeld filtered)
V	Complete	Pyridoxine hydrochloride	2 mg. pyridoxine hydrochloride in saline (steam sterilized)
VI	Complete	Nicotinic acid	3 mg. nicotinic acid in saline (steam sterilized)
VII	Complete	Inositol	4 mg. inositol in saline (steam sterilized)
VIII	Complete, but used O.M.W. salt mix instead	None	None
IX	Complete	Para-aminobenzoic acid	4 mg. para-aminobenzoic acid in saline (steam sterilized). Warmed to 90° C. to dissolve crystals; cooled and injected
IXa	Complete, but in addition a saturated solution of sulfadiazine was provided as drinking water	Para-aminobenzoic acid	Same as for Diet IX
X	Complete	2 methyl naphthoquinone (vitamin K)	2 mg. vitamin K in sterile saline; prepared by grinding in sterile mortar and pestle
XI	Complete	Choline chloride	4 mg. choline chloride in saline (steam sterilized)
XII	Complete	Biotin	2.5 mg. biotin in sterile buffered saline
XIII	Complete	Alpha-tocopherol (vitamin E)	5 mg. vitamin E in sterile mineral oil
XIV	Same as Diet VIII but dextrin substituted for sucrose	None	None
XV	Complete	Nicotinic acid and inositol	3 mg. nicotinic acid and 4 mg. inositol in saline (steam sterilized)
XVI	Complete	Thiamine hydrochloride and riboflavin	2 mg. thiamine hydrochloride in saline (filtered) and 2 mg. riboflavin in saline (steam sterilized)

*Consists of sucrose, vitamin-free casein, hydrogenated vegetable oil (Crisco), U. S. P. salt mix No. 2.

†Vitamin supplement: thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, Calcium pantothenate, nicotinic acid, para-aminobenzoic acid, inositol, choline chloride, biotin, alpha-tocopherol, 2-methyl naphthoquinone, and a vitamin A and D preparation (Vi-delta).

vitamin in solution or suspension was supplied in adequate amounts by parenteral injections, twice weekly.

In the beginning the injection was subcutaneous on the abdominal surface, but the animals getting riboflavin by this method developed sterile abscesses. Subsequently intraperitoneal injections were given. There was no evidence of peritoneal inflammation following administration by this route. The biotin solution was strongly acidic and on injection seemed to shock some of the animals temporarily, but mixing it with equal quantities of sterile buffered saline seemed to lessen this effect to some extent.

To test the possibility that the U.S.P. No. 2 salt mixture might not be adequate for hamsters, Diet VIII was devised using the salt mixture of Osborne, Mendel, and Wakeman (O. M. W.). This diet was prepared with vitamin-free casein, sucrose, a hydrogenated vegetable oil,* salt mixture, and vitamin supplement in essentially the same proportions as for Diet I. Diet VIII was also used as a control for Diet XIV in which sucrose was replaced by dextrin, a polysaccharide which is less readily attacked by bacteria of the oral cavity, in an attempt to evaluate the function of carbohydrate in the experimental diet. The basic composition of Diet VIII consisted of the following:

Sucrose	60 per cent
Vitamin-free casein	25 per cent
Vegetable oil	10 per cent
Salt mixture (O.M.W.)	4 per cent
NaCl	1 per cent

The salt mixture was composed of these substances:

$CaCO_3$	543.0 Gm.
KH_2PO_4	212.0 Gm.
KCl	112.0 Gm.
NaCl	69.0 Gm.
$FePO_4 \cdot H_2O$	20.5 Gm.
$MgCO_3$	25.0 Gm.
$MgSO_4$	16.0 Gm.
$CuSO_4$	0.9 Gm.
$MnSO_4$	0.35 Gm.
$Al_2(SO_4)_3 \cdot K_2SO_4$	0.17 Gm.
KI	0.08 Gm.

The same vitamin supplement used for Diets VIII and XIV was used for the rest of the experimental diets as well.

Procedures

Observations of the general state of health of the animals were made frequently and weights were determined about twice a week. At the end of the 100-day experimental period, the hamsters were sacrificed, the jaws of each animal were removed and stored in 70 per cent alcohol. After several days, when the remaining soft tissues had hardened enough to be easily removed, the cleaned jaws were observed with a binocular microscope (magnification of ×15 and ×20) for carious lesions. The incidence of these molar lesions was evaluated,

*Crisco, prepared by Procter & Gamble, Co., Cincinnati, Ohio.

both by the percentage of molars found carious and by a method of scoring almost identical with that of Keyes,[17] wherein the number as well as the size of cavities could be expressed in a caries index for each dietary group.

Preliminary tests were made with 9 small groups of hamsters (Table III). These tests were repeated and extended as shown in Table IV. In the preliminary tests the highest percentage of carious lesions occurred in animals on Diet IX, not containing para-aminobenzoic acid, although this substance was given parenterally. In anticipation of a similarly high incidence in the later experiments, two groups of hamsters were placed on the diet deficient in para-aminobenzoic acid, one of them having as its water supply a saturated aqueous solution of sulfadiazine (Diet IXa). (Table IV.)

TABLE III

PRELIMINARY TESTS. PERCENTAGE OF CARIOUS MOLARS IN HAMSTERS FED FOR 100 DAYS ON DIETS VARYING IN VITAMIN B CONTENT

DIET NUMBER	DIET DESCRIPTON		NUMBER AND SEX OF HAMSTERS	PERCENT OF CARIOUS MOLARS		
	BASAL DIET	VITAMIN SUPPLEMENT		MANDIBULAR	MAXILLARY	TOTAL
I	Sucrose casein vegetable oil salt mix	Complete vitamin Supplement	26 ♂♂ 22 ♀♀	20 51	26 41	23 47
II	Same as Diet I	Complete, except for riboflavin	10 ♂♂ 12 ♀♀	41 32	48 30	45 30
III	Same as Diet I	Complete, except for calcium pantothenate	14 ♂♂ 13 ♀♀	49 41	48 20	48 30
IV	Same as Diet I	Complete, except for thiamine hydrochloride	9 ♂♂ 13 ♀♀	55 47	55 24	55 36
V	Same as Diet I	Complete, except for pyridoxine hydrochloride	7 ♂♂ 7 ♀♀	86 57	90 52	88 55
VI	Same as Diet I	Complete, except for nicotinic acid	3 ♂♂ 3 ♀♀	80 72	88 88	83 81
VII	Same as Diet I	Complete, except for inositol	5 ♂♂ 3 ♀♀	30 28	63 33	46 30
VIII	Same as Diet I but O.M.W. salt mix instead	Complete vitamin supplement	6 ♂♂ 1 ♀	66 66	72 66	69 66
IX	Same as Diet I	Complete, except for para-amino-benzoic acid	3 ♂♂ 3 ♀♀	100 100	100 100	100 100

Contrary to findings in preliminary tests it became apparent early in the course of the experiment that animals on Diets VI and VII would have a relatively low dental caries incidence. Accordingly, Diet XV was prepared with both nicotinic acid and inositol deleted; similarly, Diet XVI was made to lack both thiamine and riboflavin. In both cases, however, these dietary double deficiencies were rectified in regard to the hamsters' own nutrition by intraperitoneal injections of the missing vitamins.

TABLE IV

EVALUATION OF DENTAL CARIES IN THE HAMSTERS USED IN THE PRINCIPAL STUDY

DIET	HAMSTERS	CARIOUS MOLARS			CARIES INDEX FOR EACH GROUP
		TOTAL NUMBER	AVERAGE NUMBER PER ANIMAL	PER CENT	
I	17 ♂♂	61	3.59	29.9	3.88
	31 ♀♀	124	4.00	33.3	4.40
	48 total	185	3.85	32.1	4.21
II	17 ♂♂	109	6.41	53.4	4.47
	18 ♀♀	89	4.94	41.2	2.32
	35 total	198	5.66	47.1	3.36
III	4 ♂♂	21	5.25	43.7	9.83
	12 ♀♀	46	3.83	31.9	4.18
	16 total	67	4.19	34.8	5.59
IV	13 ♂♂	92	7.08	59.0	8.05
	6 ♀♀	39	6.50	54.2	7.11
	19 total	131	6.89	57.4	7.75
V	12 ♂♂	83	6.92	57.6	5.57
	8 ♀♀	31	3.88	32.3	3.75
	20 total	114	5.70	47.5	4.84
VI	7 ♂♂	16	2.30	19.0	3.64
	21 ♀♀	51	2.43	20.2	1.59
	28 total	67	2.39	19.9	2.10
VII	23 ♂♂	123	5.35	44.5	3.63
	17 ♀♀	79	4.65	38.7	3.99
	40 total	202	5.05	42.0	3.79
VIII	19 ♂♂	80	4.21	35.1	5.95
	30 ♀♀	173	5.77	48.0	7.27
	49 total	253	5.16	43.0	6.76
IX	25 ♂♂	91	3.64	30.4	3.07
	39 ♀♀	192	4.92	41.0	3.69
	64 total	283	4.42	36.9	3.45
X	15 ♂♂	138	9.20	76.6	16.62
	26 ♀♀	240	9.23	76.6	12.34
	41 total	378	9.22	76.6	13.91
XI	24 ♂♂	234	9.75	84.5	12.13
	25 ♀♀	199	7.96	66.1	8.35
	49 total	433	8.84	73.8	10.21
XII	4 ♂♂	18	4.50	37.5	3.70
	15 ♀♀	53	3.53	29.5	3.58
	19 total	71	3.74	31.1	3.61
XIII	24 ♂♂	181	7.54	62.9	3.84
	21 ♀♀	102	4.86	40.4	2.08
	45 total	283	6.29	52.2	3.02
XIV	15 ♂♂	52	3.47	28.8	3.71
	14 ♀♀	34	2.43	20.3	2.44
	29 total	86	2.97	24.7	3.09
XV	13 ♂♂	109	8.38	69.7	16.70
	33 ♀♀	323	9.79	81.5	1.91
	46 total	432	9.39	78.0	6.09
XVI	27 ♂♂	152	5.63	46.8	3.16
	19 ♀♀	133	7.00	58.3	3.76
	46 total	285	6.19	51.6	3.41
Special "Purina" meal	15 ♂♂	22	1.47	12.2	1.39
	20 ♀♀	7	0.35	2.9	0.05
	35 total	29	0.83	6.9	0.62
IXa (with sulfadiazine water)	22 ♂♂	139	6.32	52.7	2.55
	31 ♀♀	209	6.42	56.4	3.02
	53 total	348	6.57	54.7	2.82

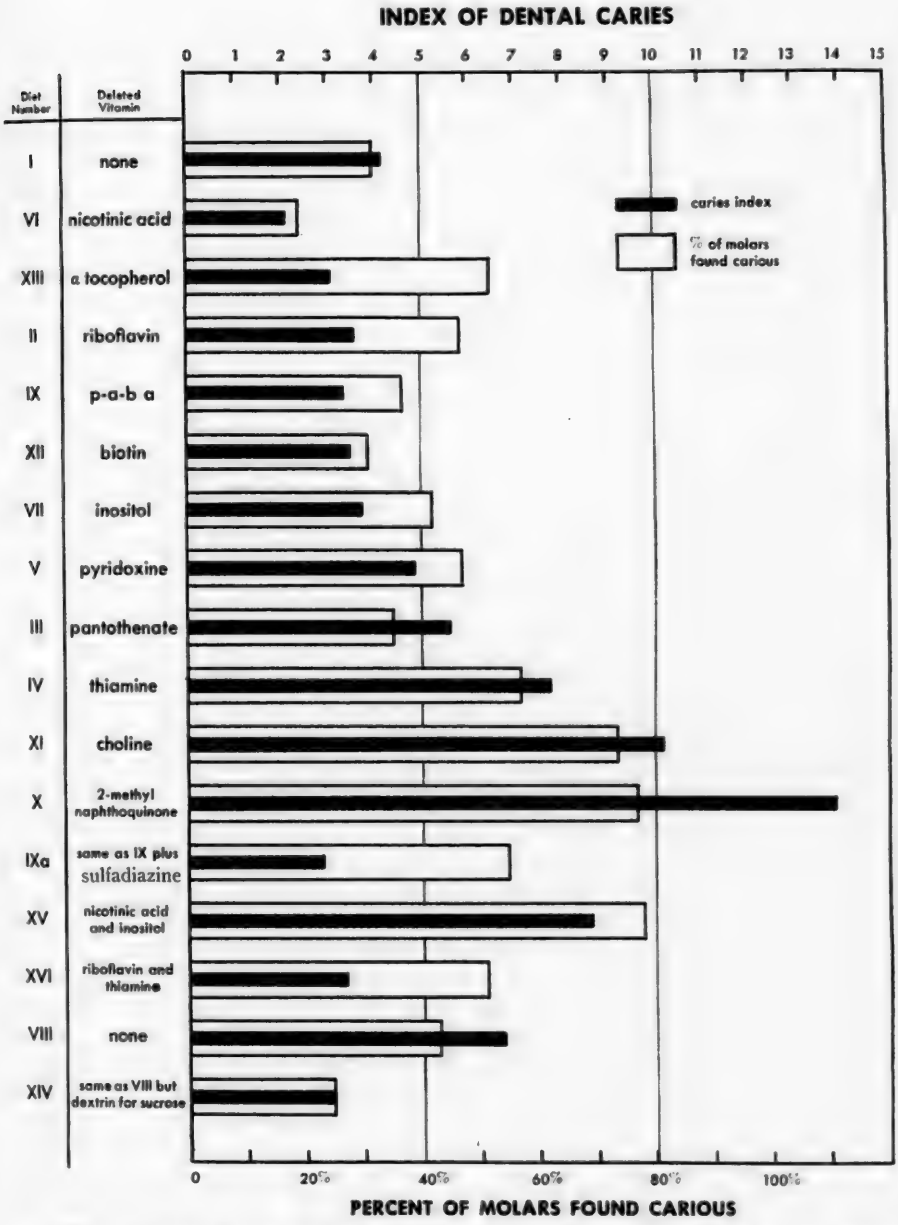

Fig. 1.—Graphic representation of the dental caries incidence for the various dietary groups of hamsters used in the principal study expressed in percentage of molars found to be carious and also by means of the caries index.

RESULTS

The distribution by diet of 682 hamsters that survived the experimental feeding period of 100 days is shown in Table IV. Also shown are the totals, averages, and percentages of the molars becoming carious as well as the caries index for each experimental group. The sexes, having been distributed among the dietary groups at random, showed a difference in susceptibility to caries. As had been reported earlier,[14] the males were somewhat more susceptible as a whole, but this fact did not alter appreciably the percentage of carious molars found nor the caries index of any of the dietary groups with one exception.

TABLE V

NUTRITIONAL REQUIREMENTS OF THE HAMSTER
(AS REPORTED IN THE LITERATURE)

VITAMINS INVESTIGATED	DEFICIENCY SIGNS AND SYMPTOMS	VITAMINS CONSIDERED ESSENTIAL AND NONESSENTIAL (NUMBERS INDICATE REFERENCES)	
		DAILY MAINTENANCE DOSE	+ ESSENTIAL − NON-ESSENTIAL
Thiamine	Polyneuritis in 12 days	3 μg. orally (25)	+(25), +(26), +(28)
Riboflavin	Perioral dermatitis and diarrhea; weight loss and death in 20 days	4 μg. orally (25)	+(25), +(26), +(28)
Pyridoxine	Acrodynia-like perioral dermatitis in 12 days; loss of weight, muscular weakness, and death in 24 days	3 μg. (25) 50 μg. (30)	+(25), +(26) +(28), +(30)
Pantothenic acid	Perioral red crusting; loss of weight and death in 20 days	parenterally, 15 μg. (25)	+(25), +(26), +(28)
Nicotinic acid	Rough, stringy fur, alopecia, stomatitis, weight loss, and death (25) Vitamin necessary only for successful lactation (28)	100 μg. (25)	+(25), −(26), −(28)
Para-amino-benzoic acid	None, if biotin is given	Undetermined	+?(26), −(28)
Inositol	None, if biotin is given. Only necessary for delivery of living young	Undetermined	+?(26), −(28)
Choline	None; only necessary for successful lactation	None	−(28)
Biotin	Cheilosis and poor growth, death 3 to 5 weeks (26)	1 μg. (26)	+(26), −(28)
Vitamin C	None	None	−(26), −(27)
Vitamin D	Typical rickets, but diet must be low in phosphorus as well as dificient in vitamin D	Undetermined	+(29)
Vitamin E	Gradual growth diminishment, terminal incoordination, excitation and shock with death	Therapeutic dose: 5 mg.	+(28)
Vitamin K	Arrested growth; hemorrhage in peritoneum and sinuses. Spontaneous recovery after 3 to 7 weeks possibly due to bacterial synthesis of vitamin K in intestines	Undetermined	+(28)

A graphic representation of the caries incidence for the various dietary groups is presented in Fig. 1. The most marked effects were observed when choline or vitamin K were removed from the diet. In these two groups of

hamsters the caries indices were, respectively, about 2.5 and 3.0 times as great as that of the control group. In the case of Diet X, lacking vitamin K, 76.6 per cent of the molar teeth of 15 male and 26 female animals studied had carious lesions, and an index of 13.91 as compared to the control Diets I and VIII whereon 32.1 per cent and 43.0 per cent, respectively, of the molars showed caries and the caries index was 4.21 and 6.76, respectively. The only dietary group which showed any marked decrease in dental caries was the one deficient in nicotinic acid. In this group the caries index was reduced to about one half that of the control group.

When a deficiency of para-aminobenzoic acid in the diet was supplemented by sulfadiazine in the water (designated as Diet IXa) the number of molars affected remained high but the caries index decreased. This may raise the question whether the drug inhibited the bacteria responsible for continuing the carious process in the dentin. A similar effect was noted when vitamin E was omitted from the diet (designated as Diet XIII) and, to a lesser degree when riboflavin and thiamine were both deleted (as in Diet XVI). On the other hand, the double deficiency of nicotinic acid and inositol seemed to increase the caries index considerably. Since these results are rather inconclusive, further careful study of much larger groups with double and multiple deficiencies seems desirable.

Substitution of dextrin for sucrose in the basic diet resulted in a decrease in the caries index by about 50 per cent.

The group of hamsters fed on the commercial meal which was used for the stock colony had exceedingly little dental caries, despite the fact that this feed was low in fluorides, the abundant presence of which is considered to be caries inhibitory. No evidence has been obtained to indicate whether this result may be due either to absence of a stimulating factor or presence of an inhibitory factor other than fluorides.

The experimental diets were not entirely adequate for growth as may be seen by comparing the weight curves for animals on the commercial, low-fluoride diet with those for hamsters in the two control groups, Diets I and VIII (Fig. 2). The animals were also somewhat abnormal in general appearance, in part because of the matting of their fur by the fat in the food with which the animals would make body contact. In several dietary groups a whitening of the usually brown dorsal fur was noted in the early part of the experimental period. This effect was observed particularly in animals on Diet III which received their calcium pantothenate by injection, and also in some on Diet II, but it was not a constant finding in these groups nor in any other. These apparent nutritional deficiencies cleared up spontaneously in each case, as if a missing factor had been eventually supplied. Such a nutritional sequence suggests the possibility of a change in the intestinal flora or in the synthesizing ability of some bacteria composing the flora.

DISCUSSION

Even a cursory review of the literature on dental decay reveals that considerable indirect evidence has been amassed to indicate some etiological relation-

ship between the dietary intake and the oral bacterial flora on the one hand and dental caries on the other. It has been shown repeatedly in man that there is some quantitative correlation of certain oral acidogenic and aciduric bacteria with amount of tooth decay.[18, 19, 20] Similar findings in the albino rat have been reported by one of us[21, 22, 23] in a systematic study of the oral bacterial flora at various stages of dental caries development in rat molars. One of the reports on the hamster study in this laboratory[15] showed that lactobacilli and aciduric streptococci tend to increase considerably in number with an increase in the dental caries incidence. Although some relationship between the presence of certain oral bacteria and tooth decay seems evident, bacteria per se are not to be considered as independent entities in the dental caries process. They act rather as potential etiological agents, active only when there are present suitable substrates which can provide the substances essential for bacterial growth and acid production.

In planning the exploratory experiments here reported, it was considered plausible that if certain growth accessory substances essential for bacterial nutrition could be substantially reduced in quantity in the oral environment, some of the bacteria composing the oral flora presumably would be unable to attain their optimal growth or to produce their normal amount of organic acid. Such a reduction in acid formation in the depths of the molar fossae and sulci could conceivably lead to a reduction in the amount of dental caries.

It has been conjectured that vitamin B components as well as other vitamins are apparently essential growth factors for some oral lactobacilli and for some time it has been known that the presence of such vitamins in media in the form of yeast extract would enhance the growth of almost all such microorganisms.

The experiments here reported may be looked upon as a study of bacterial growth in vivo using the oral cavity of the hamster as the immediate environment. They represent tests of the effects of thiamine, riboflavin, pyridoxine, calcium pantothenate, nicotinic acid, inositol, para-aminobenzoic acid, choline, biotin, 2-methyl naphthoquinone, and alpha-tocopherol upon the caries-producing capacity of the normal, mixed oral flora, that is, tests to determine, if possible, which vitamin constituents of the diet are essential for bacterial acid production in the mouth. The degree of acid production occurring on susceptible tooth surfaces might be measured grossly by the amount of decay in the hamster's molars. However, since most, if not all, of these nutritional accessory substances are required in the metabolic processes of the hamster (Table V), whenever one of them was omitted from the diet it was provided by parenteral injection. Unless the injected vitamin was subsequently concentrated in the oral secretions, or the nutritional needs of the bacteria were otherwise supplied, this procedure should have produced effects attributable to reduced availability of the test substances to the bacterial flora of the tooth surfaces.

The findings as summarized in Table IV and Fig. 1, although not marked, seem to show some measurable effects of reduced vitamin content upon the ex-

tent of caries activity. Omission from the diet of both nicotinic acid and inositol, of thiamine, of choline, or of 2-methyl naphthoquinone (vitamin K) resulted in increased caries. The role of vitamin K in the dental caries process is not very clear. Acid formation in the saliva has been found to be reduced by the presence of synthetic vitamin K and a few related quinone compounds[31] while clinical studies seem to show that 2-methyl-1, 4-naphthoquinone (synthetic vitamin K) used in chewing gum inhibits dental caries in man.[32]

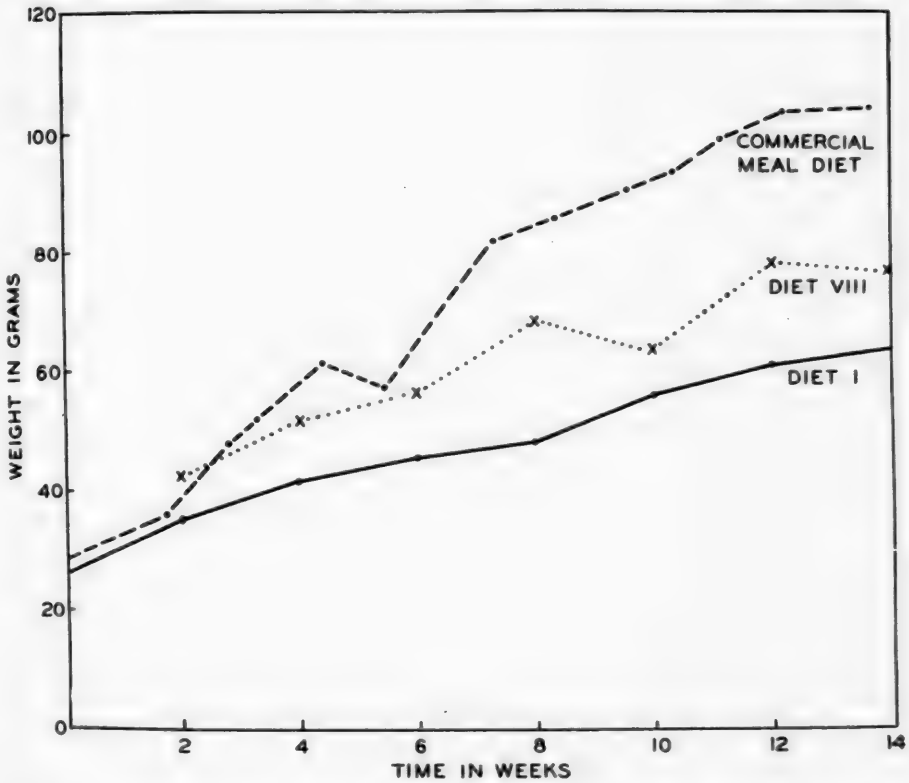

Fig. 2.—Comparative growth curves of hamsters fed experimental diets complete in vitamin supplements and of those fed the low-fluoride commercial meal diets.

Choline chloride, recognized as a growth factor in some animals, has not been found essential to the nutrition of the hamster except for successful lactation[28] nor has it been reported to be an essential growth factor for oral bacteria associated with dental caries. Its apparent effect on the development of caries in the hamster is not clear and should be further investigated.

Although hamsters fed the diet deficient in thiamine showed somewhat more tooth decay than control animals, thiamine according to the in vitro studies of Weisberger and Johnson[24] was found to be an essential growth factor for the

lactobacillus strain studied by them. On the other hand, Koser and Fisher,[33] in a study of 26 strains of oral lactobacilli, found that thiamine was needed by only about one third of the cultures.

When nicotinic acid was absent from the diet (Diet VI), the amount of dental caries was less than half that found in the control Diets I and VIII. In this connection it is interesting to note that Weisberger and Johnson also showed nicotinic acid to be essential for growth of their oral strain of lactobacillus. Likewise, Koser and Fisher reported nicotinic acid was needed by all 26 of their oral strains studied. This would seem to be a significant finding if it were known that all microorganisms associated with dental caries required nicotinic acid and if other dietary deficient groups of animals showed correlative trends. Inositol is not known to be a growth factor for oral bacteria and its absence from the diet seemed not to influence the amount of dental decay in the hamsters. Yet, when the double deficiency of nicotinic acid and inositol is considered, the amount of dental caries was found to be as high as when thiamine or choline were removed from the diet.

The caries index was decreased in animals fed the diet deficient in riboflavin (Diet II) although the number of molar teeth with carious lesions was larger than in the control groups on the complete diet. The same result was obtained when riboflavin deficiency was combined with a deficiency in thiamine (Diet XVI). These results combined with the findings that the caries index was twice as high in animals on a diet which contained riboflavin but was deficient in thiamine (Diet IV) suggest that riboflavin is utilized by the caries-producing flora of the hamster and that its presence is conducive to caries development. Riboflavin was found to be required by the majority of oral lactobacilli isolated from patients with dental caries as observed by Koser and Fisher[33] in their in vitro study. But by contrast, the observation of Weisberger and Johnson[24] indicated that riboflavin was not essential to the growth of the one strain of oral lactobacillus investigated. It must be borne in mind, however, that the oral cavity of rodents as well as of man contains a very complex bacterial flora, that more than one bacterium may be involved in caries production, and that the over-all nutritional requirements of the caries-associated flora may well be different from that of one isolated microorganism observed in vitro.

The amount of dental caries found in hamsters maintained on diets deficient in pyridoxine, pantothenate, para-aminobenzoic acid, and biotin did not seem to differ to a significant degree from that found in animals on the two control diets.

Absence of alpha-tocopherol (vitamin E) did not inhibit the initiation of carious lesions, but apparently had a retarding effect on the progression of such lesions as indicated by the low caries index. In several groups the total number of molar teeth becoming carious (Diets II, IXa, XIII, and XVI) was high in comparison with the total quantity of caries as indicated by the caries index. This suggests the possibility of a kind of bacterial ecological succession in the production of the lesion. Hence, the absence of certain vitamins essential to microorganisms which are important only in the later stages of the dental caries

process may be the explanation for the lessened amount of dentin destruction in these groups. Earlier observations in this laboratory[22, 23] support such a sequence of bacterial succession. The flora of the enamel surfaces of the molar teeth of rats exhibited a progressive increase in the proportion of lactobacilli as well as in alpha streptococci while caries was developing, whereas the aerobic flora of large dentinal lesions consisted almost entirely of streptococci. Moreover, bacterial cultivation from hamsters with carious lesions[15] also tended to portray a similar picture in so far as animals with a low caries index showed lactobacilli to predominate, but where the carious destruction was very great, streptococci outnumbered the lactobacilli cultivated.

Assuming the possibility of reducing the oral flora, sulfadiazine was added to the drinking water of hamsters on a diet lacking para-aminobenzoic acid (Diet IXa). It must be considered that this drug might also exhibit antagonistic action on the intestinal bacteria, inhibiting the synthesis of para-aminobenzoic acid as well as other growth accessory substances which might eventually find their way to the oral cavity. The findings reported here show a higher, rather than a lower, percentage of carious molars although the caries index is not very different from that in para-aminobenzoic acid-deficient animals not receiving the sulfa drug (Diet IX).

Hamsters maintained on Diet XIV, in which dextrin was substituted for sucrose, showed half as much carious destruction as the animals receiving sucrose (Diet VIII). This seemed significant in view of the almost invariable in vitro finding that the polysaccharide, dextrin, is much less readily utilized by oral bacteria for acid production than is the disaccharide, sucrose, Similarly, it has been shown by Schweigert, Shaw, Phillips and Elvehjem[34] that replacing 75 per cent of the sucrose with fine dextrin reduced both the incidence and extent of caries in the cotton rat.

As previously noted, this study was planned as a series of exploratory dietary tests. Most of the results have been equivocal or uncertain. There is some indication, however, that caries is increased in the absence of either choline, thiamine, or vitamin K (2-methyl naphthoquinone). Although deficiencies in nicotinic acid and inositol, separately, did not result in increased caries, there was a considerable increase when both were removed from the diet. Deletion of nicotinic acid alone seemed to decrease the caries. Interpretation of the results is confused by a number of factors. For example, the normal bacterial flora in these rodents is extremely complex and has never been completely studied. When such animals are maintained on special diets, the substrate of the oral cavity changes, the presence of carious lesions in the teeth again changes the oral environment and consequently the oral flora. Additionally, coprophagy could be only minimized, not eliminated. To further complicate matters, it should be mentioned that among bacteria deprived of certain vitamins essential for their growth, there may exist certain variants capable of synthesizing the missing vitamin. Under conditions of laboratory cultivation it has been demonstrated that a strain of propionibacterium (belonging to the same tribe as lactobacilli) could be "trained" to synthesize appreciable quantities of thiamine.[35, 36]

Moreover, in a report by Koser and Wright[37] it was found that from stock cultures of dysentery bacilli which require nicotinamide for growth variants were obtained capable of growth without the vitamin. It was therefore suggested that apparently nicotinamide and coenzyme or some substance physiologically equivalent must be synthesized by these variants. If such adaptations by bacteria can occur in laboratory cultivation, there is reason to suspect that it may also occur in such a natural environment as the oral cavity.

SUMMARY

Based on the assumption that certain oral bacterial are important in the development of dental caries, this investigation concerned itself with the environment of the oral cavity in the Syrian hamster. The factors considered were: (A) the nutritional substrate provided by the ingested food, (B) the bacterial flora subsisting on this substrate, and (C) the molar teeth subjected to carious destruction presumably by the action of the bacteria. Partly synthetic experimental diets containing 11 vitamins in measured amounts, with 1 or 2 of these vitamins being omitted from each test diet, were fed to 682 hamsters distributed into 18 different dietary groups for a 100-day period. During this time, the animals were provided with adequate amounts of the missing vitamins semiweekly by intraperitoneal injection for their own nutrition. As a plausible measure of the degree to which certain oral bacteria were retarded or accelerated in their growth and metabolic activities, the extent of the carious lesions in the hamsters' molar teeth were evaluated.

The trends observed in this study were the following:

1. When vitamin K was absent in the diet, hamsters showed the greatest amount of dental caries.

2. The animals on a diet deficient in choline chloride developed about twice as much caries as animals in the control groups.

3. With the omission of nicotinic acid, the amount of caries was reduced to about one-half that of the control animals.

4. Animals receiving neither nicotinic acid nor inositol showed a higher degree of dental caries than the control animals.

5. Absence of vitamin E did not seem to prevent lesions from occurring but apparently retarded their progress.

6. Substitution of dextrin for sucrose caused a 50 per cent decrease in molar destruction by dental caries.

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PRELIMINARY STUDIES OF THE CARIES INHIBITING POTENTIAL AND ACUTE TOXICITY OF SODIUM MONOFLUOROPHOSPHATE

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LITTLE is known concerning the forms in which fluorine may be administered to be effective against dental decay. All studies to date have dealt with compounds which dissociate to some extent in water to yield the fluoride ion. It was of interest, therefore, to test the ability to inhibit caries activity and the acute toxicity of a complex fluoride, monofluorophosphate, which does not yield fluoride ions in aqueous solution.

The sodium salt of monofluorophosphate used in this study may be characterized as follows:‡ It is soluble to the extent of about 25 Gm. in 100 Gm. of water at room temperature yielding a solution with a pH of 7.0 to 7.5. The salt yields the $PO_3F^=$ ion which is stable at ordinary temperatures in neutral or slightly alkaline solutions. In acid solution the complex slowly hydrolyzes to orthophosphate and fluoride. The $PO_3F^=$ ion does not give the analytical reactions of fluoride or of phosphate ions and gives few insoluble salts. The calcium salt, for example, has a solubility of about 1 Gm. per 100 Gm. of water at 20° C., a value several hundred times greater than the solubilities of calcium fluoride or calcium phosphate.

EFFECT OF SODIUM MONOFLUOROPHOSPHATE ON EXPERIMENTAL DENTAL CARIES IN SYRIAN HAMSTER

Experimental

Sixty Syrian hamsters (*Cricetus auratus*), 29 to 42 days of age from 11 litters, were divided by litter-mate distribution into 3 groups of 10 males and 10 females each. All groups received the same high carbohydrate caries-producing diet[1] ad libitum and were housed in screen bottom cages. The groups differed only in the drinking solutions supplied ad libitum:

Group I received distilled water; Group II received distilled water containing 40 ppm fluoride as sodium fluoride, and Group III received distilled water containing 40 ppm fluoride as sodium monofluorophosphate (303 ppm). In order to preclude the possible decomposition of the dilute solution of sodium monofluorophosphate, a 1.519 per cent stock solution was prepared and stored at 6° C. Every second day a 20 c.c. portion of this solution was diluted to 1,000 c.c. with distilled water and placed into cleaned drinking bottles of Group III.

The duration of the experiment was 113 days.

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‡These data kindly supplied by the Research Laboratory of the Ozark-Mahoning Co. of Oklahoma.

Results

The average weights of the animals at the time of sacrifice were as follows:

GROUP	MALES	FEMALES
I (H_2O)	92	87
II (NaF)	89	87
III (Na_2PO_3F)	95	88

Three animals died during the course of the experiment; 2 in the male control group and 1 in the male sodium fluoride group. The cause of death was not ascertained.

No significant difference in gross appearance was noted among the animals of the various groups.

Table I gives in detail the caries experience of each of the animals. The data are summarized in Table II.

TABLE I

CARIES EXPERIENCE OF MALE AND FEMALE SYRIAN HAMSTERS DRINKING: DISTILLED WATER, 40 PPM F FROM NeF, 40 PPM F FROM Na_2FPO_3

FEMALES						MALES				
	NO.	MA*	CN†	AA‡	TS§		NO.	MA*	CN†	AA‡	TS§
						Control					
	23	11	21	14.0	30.8		2	12	16	29.3	78.5
	17	12	20	6.5	13.0		43	12	17	26.0	60.8
	6	10	14	6.5	9.5		57	1	1	0.3	0.3
	9	12	20	10.3	16.5		22	11	16	37.3	92.8
	1	8	8	3.8	8.3		50	8	13	18.8	40.0
	49	6	7	4.8	9.8		15	8	9	17.8	47.0
	26	12	21	21.8	45.8		37	10	17	31.3	86.0
	45	11	16	19.5	44.3		34	11	16	47.5	102.3
	55	7	12	8.3	21.3						
	12	11	20	16.3	21.5						
Avg.		10.0	15.9	11.2	22.1	Avg.		9.1	13.1	26.0	63.4
						NaF					
	19	4	6	1.0	1.0		32	1	1	1.5	3.0
	11	6	9	2.3	2.3		29	5	5	0.3	0.3
	14	5	5	4.8	10.3		46	5	6	2.0	4.8
	25	4	4	2.8	5.8		54	3	3	0.5	0.5
	33	8	15	4.5	5.0		36	3	3	0.3	0.3
	8	7	9	2.3	2.3		21	3	4	1.0	1.0
	42	4	4	1.5	2.3		59	2	2	0.3	0.3
	48	6	7	2.0	2.0		13	4	5	3.0	8.0
	5	8	9	6.5	6.5		40	6	7	1.3	1.8
	53	5	5	1.3	2.3						
Avg.		5.7	7.3	2.9	4.0	Avg.		3.5	4.0	1.2	2.2
						Na_2FPO_3					
	47	8	12	2.3	3.3		52	3	3	0.5	0.5
	41	6	7	2.0	3.0		60	2	2	0.3	0.3
	4	12	19	5.0	5.0		35	3	3	0.0	0.0
	51	6	6	0.5	0.5		58	3	3	0.0	0.0
	56	1	1	0.0	0.0		39	3	3	0.3	0.3
	28	4	6	2.8	3.3		44	6	10	1.0	1.5
	24	7	11	4.0	6.5		3	6	11	0.5	0.8
	10	6	7	1.8	2.3		31	8	9	2.5	4.3
	18	11	18	4.3	5.8		20	4	4	0.8	1.0
	7	7	10	6.0	10.8		27	6	6	2.8	5.8
Avg.		6.8	9.7	2.9	4.0	Avg.		4.4	5.4	0.9	1.4

*Molars affected.
†Number of cavities.
‡Total area of teeth affected.
§Total caries score (area × depth).

TABLE II
AVERAGE CARIES EXPERIENCE OF ANIMALS DRINKING:
DISTILLED WATER, 40 PPM F FROM NaF, 40 PPM F FROM Na_2FPO_3

GROUP	NO. OF ANIMALS	MOLARS AFFECTED	NO. OF CAVITIES	AREA AFFECTED	TOTAL CARIES SCORE
Males					
I (H_2O)	8	9	13	26	63
II (NaF)	9	4	4	1	2
III (Na_2PO_3F)	10	4	5	1	1
Females					
I (H_2O)	10	10	16	11	22
II (NaF)	10	6	7	3	4
III (Na_2PO_3F)	10	7	10	3	4

In the females the number of molars affected and the number of cavities were reduced approximately 30 to 50 per cent by fluoride as sodium monofluorophosphate or sodium fluoride. In the same animals the total area of the teeth affected by caries and the total caries scores were reduced about 70 and 80 per cent, respectively, for both compounds.

The number of molars affected and the number of cavities was reduced in the male hamsters about 55 and 70 per cent, respectively, for both compounds, while the total area of the teeth affected and the total caries scores were reduced approximately 95 per cent.

Discussion

The close agreement of the final body weights and the absence of any obvious differences in appearance of the animals indicate that the materials in the concentration employed had no marked effect on the well-being of the animals.

It appears from the data on caries experience (Tables I and II) that the addition to the drinking water of 40 ppm of fluoride either as F^- or $FPO_3^=$ will markedly decrease the destruction of tooth substance by caries in the Syrian hamster. The best quantitative expression of this difference in tooth destruction is obtained by contrasting the total caries scores. It will be seen that while the hamsters drinking water containing monofluorophosphate averaged total caries scores of 1.4 for males, 2.2 for females, and the males and females given NaF averaged 4, the control males and females averaged scores of 63 and 22, respectively. It is impossible to say from this work whether fluoride as monofluorophosphate is more effective than fluoride as sodium fluoride because the level of 40 ppm of fluoride employed gave very nearly maximal protection with each compound. By comparing the caries experience at lower fluoride concentrations any differences in potency against dental caries might appear.

A recalculation of the amount of fluoride administered as fluorophosphate based upon more exact data concerning the purity of the compound* indicates that a level of 39 ppm fluoride was obtained in the drinking water of the sodium monofluorophosphate group. Of the total 39 ppm, 7 ppm F were contributed by the 20 per cent of impurities contained in the test sample. How-

*Wayne E. White. Personal communication.

ever, the 7 ppm of fluoride contributed by the impurities would not, if given as NaF, have produced the caries reduction observed in these experiments.[3]

ACUTE TOXICITY OF SODIUM MONOFLUOROPHOSPHATE

Experimental

Intraperitoneal Injection.—One hundred and five albino rats weighing 200 to 300 grams were divided into 13 groups of from 5 to 15 animals. Graded doses of from 74 mg./kg. to 465 mg./kg. of sodium monofluorophosphate as a 5 per cent aqueous solution were administered intraperitoneally and the number dead in 24 hours noted. From these data the predicted LD50 was calculated by use of the Winthrop logarithmic-probit graph paper.[4]

Oral Administration.—Forty-five fasted albino rats weighing 200 to 300 grams were divided into 9 groups of 5 animals. Single doses of from 263 mg./kg. to 1323 mg./kg. of the 5 per cent aqueous solution were given by stomach tube. The percentage mortality observed in 24 hours was calculated and the predicted LD50 obtained by graphical analysis.

For comparison, the acute oral toxicity of sodium fluoride as a 2.5 per cent solution for 200-300 gram rats was also obtained. 98 rats were used and graded oral doses of from 22 to 288 mg./kg. were given in single injections by stomach tube.

Results

The predicted LD50 of sodium monofluorophosphate by intraperitoneal injection was found to be 220 ± 32 mg./kg.* Compared to the reported[5] LD50 of 24 mg./kg. for sodium fluoride (as a 2 per cent aqueous solution) in 200 to 300 gram rats, sodium monofluorophosphate is about 8 to 9 times less toxic.

The predicted LD50's of sodium monofluorophosphate and sodium fluoride by oral administration were found to be 570 ± 68 mg./kg. and 80 ± 5 mg./kg., respectively. Na_2PO_3F is one-eighth as toxic as NaF.

The results are summarized in Table III which contrasts the toxicities of sodium monofluorophosphates and sodium fluoride on both the basis of milligrams of compound per kilogram of body weight and milligrams of fluoride per kilogram of body weight.

TABLE III

ACUTE TOXICITY OF Na_2PO_3F AND NAF IN 200 TO 300 GRAM RATS

DOSE	Na_2PO_3F (MG./KG.)	NAF (MG./KG.)
Intraperitoneal LD50	220	24
Oral LD50	570	80
On Basis of Fluoride Content		
DOSE	F AS Na_2PO_3F (MG./KG.)	F AS NAF (MG./KG.)
Intraperitoneal LD50	29	10
Oral LD50	75	32

Discussion

Sodium monofluorophosphate is considerably less toxic to rats than is sodium fluoride. On the basis of fluoride content, which is a more pertinent

*Standard error.

comparison for dental purposes, the complex fluoride apparently is 2.5 to 3 times less toxic than the free ionic form indicating that neither in the tissue fluids (intraperitoneal injection) nor in the gastrointestinal tract (oral dose) is the complex appreciably hydrolyzed.

CONCLUSIONS

When administered at a level of 40 ppm of fluoride in the drinking water of Syrian hamsters, sodium monofluorophosphate and sodium fluoride caused comparable and nearly maximal reductions in the destruction of tooth substance by dental decay.

Sodium monofluorophosphate was found to be 7 to 8 times less toxic in 200 to 300 gram rats than was sodium fluoride. Calculated on the basis of fluoride content the complex fluoride is 2.5 to 3 times less toxic than the free ionic form.

In view of the fact that the monofluorophosphate complex was apparently not appreciably hydrolyzed in the animal body, one may infer that fluorine does not have to be in a free ionic form to inhibit dental decay in the Syrian hamster.

The authors wish to thank Dr. Elliot A. Maynard for his assistance in obtaining the toxicity data.

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THE EVANSTON DENTAL CARIES STUDY

VI. A Comparison of the Prefluoride With the Postfluoride Caries Experience of 6-, 7-, and 8-Year-Old Children in the Study Area (Evanston, Ill.)

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THE DENTAL Caries Study was formulated in 1945 with the primary objective of determining whether the addition of sodium fluoride to a public water supply would have a retarding effect on dental caries similar to that found where a fluoride is a natural constituent of the water.

These data will lend themselves for a number of other comparisons. Some other objectives are: (1) Comparison of the dental caries experience of male school children with that of female school children. (2) To compare the dental caries experience of white with that of Negro school children. (3) Comparison of the effectiveness of finding dental caries by clinical examination with that by x-ray examination. (4) Comparison of the dental caries experience of school children who frequently use sweets, such as jam, jelly, candy, gum, soft drinks, cookies, etc., to that of children who rarely use them. (5) Comparison of dental caries experience of children with good oral hygiene to that of children with poor oral hygiene. (6) Comparison of dental caries experience of children according to salivary counts for lactobacillus. (7) Measurement of the effect of simple occlusal pit and fissure caries in permanent molars on future dental caries experience in these teeth.

Blayney and Tucker[1] described the mechanism of the Evanston Dental Caries Study and postulated that a period of 15 years would allow for observations and comparisons of a group of children through the period of tooth development, with the exception of the third molars. Martin[2] reported upon the fluorine content of foods available in the Evanston markets, the fluorine content of bone, and of extracted deciduous and permanent teeth.

METHOD

Dental examinations made prior to February, 1947, in the study area and before February, 1948, in the control area provide the base line data. The original observations, or base line examinations, were made on 4,375 school children in the study area and 2,493 children in the control area. The 1946 observations reported here are for 1,991 children of the 6-, 7-, and 8-year-old age group from the base line examinations of the study area. The 1948 observations in the study area were made on 2,034 children of the 6-, 7-, and 8-year-old age group after having been exposed to fluorided drinking water from 12 to 22 months at the time of the dental examinations.

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Sodium fluoride was added to the drinking water of Evanston, Ill., in February, 1947. Dental examinations, with full mouth radiographs, were resumed in the study area 12 months later. The reason for the difference in number of months of exposure to fluorided water is a matter of the time at which a child is examined. The 1948 examinations were spread over a period of 1 year. Those students examined at the close of 1948 were exposed to fluorided water longer than those students examined at the beginning of the year. All students were exposed to the water at least 12 months.

While the study has been so planned as to measure every variable that might exert an influence and obscure the findings, it is necessary to test the effect of every variable statistically. In order to be able to generalize from our findings, we must be certain that any such variables as affect caries experience are represented in our study to the same extent as in the population. Before drawing any ultimate conclusions, we will, therefore, correct our data in such a manner as to include only those groups of children which are representative of the population, with respect to dental caries experience. We feel that this precaution is necessary to allow the ultimate findings to be considered valid and reliable. It is generally admitted by many investigators that valid data regarding the caries rate in a study program such as this one would not be available until a period of at least 5 years had elapsed after the addition of sodium fluoride to the communal drinking water. We are hopeful that the trend will be established by that time. However, a much longer period will be required to ascertain the full effects.

FINDINGS

We do not anticipate finding significant changes in the caries prevalence rate for some time. Nevertheless, we are eager to make comparisons as early and as frequently as possible to observe and recognize such changes that may be taking place.

To best find an indicative measure of the trend for the caries rates in the pre- and postfluoride periods to date, we have chosen to report on: (1) the decayed, extracted, and filled rate of the deciduous teeth; (2) the number of children with deciduous dentitions immune to dental caries at the time of examination; (3) the decayed, missing, and filled rate of the permanent teeth; (4) the caries experience of the occlusal surfaces of the first permanent molar teeth; (5) the caries rate of the maxillary and mandibular anterior permanent teeth; and (6) the precarious lesions found on surfaces of all permanent teeth present in the mouths of children in these age groups.

In Table I are found the number of children examined in each age group, and the decayed, extracted, and filled rate per hundred children in 1946 and in 1948. It is apparent that the 6- and 8-year-old children have a higher decayed, extracted, and filled rate in 1948 than in 1946. The 7-year-old age group indicated a slight decrease per hundred children. As these deciduous teeth were in the mouth and fully erupted before the addition of sodium fluoride to the water supply, little action on these teeth could be expected. The fluctuations should, therefore, be considered to be due to normal fluctuations from year to year.

TABLE I

DECAYED, EXTRACTED (DUE TO CARIES), AND FILLED DECIDUOUS TEETH PER HUNDRED CHILDREN. PREFLUORIDE EXAMINATIONS AND 12 TO 22 MONTHS' POSTFLUORIDATION OF THE STUDY AREA WATER SUPPLY

	AGE (YRS.)				PROBABILITY OF DIFFERENCE DUE TO CHANCE*
	6	7	8	6-7-8	
Prefluoride, 1946					
Exams.	461	759	771	1991	
R.P.H.†	482.86	550.06	577.17	545.00	
					0.005
Postfluoride, 1948					
Exams.	756	838	440	2034	
R.P.H.	507.80	541.05	620.22	577.28	

*The probability of this difference being due to chance is 0.005. Any probability less than 0.05 is conventionally considered significant.

†R.P.H. is used to indicate rate per hundred throughout tables.

The deciduous dentition immunity rate per hundred children at the time of the dental examination showed a decrease for the 6- and 7-year-olds in 1948 and an increase for the 8-year-olds. However, as the difference in rates per hundred children was so slight, taking the 6-, 7-, and 8-year-olds together, it is not considered significant (Table II).

TABLE II

IMMUNE DECIDUOUS DENTITIONS AT TIME OF DENTAL EXAMINATION. RATE PER HUNDRED CHILDREN

	AGE (YRS.)				PROBABILITY OF DIFFERENCE DUE TO CHANCE*
	6	7	8	6-7-8	
Prefluoride, 1946					
Exams.	461	759	771	1991	
R.P.H.	21.04	11.33	6.09	11.55	
					0.68
Postfluoride, 1948					
Exams.	756	838	440	2034	
R.P.H.	15.87	8.71	8.18	11.26	

*The probability of this difference being due to chance is 0.68. Any probability less than 0.05 is conventionally considered significant.

The permanent teeth rate per hundred children was lower in the postfluoride area (1948) than in the prefluoride area (1946) (Table III). The caries rate per hundred children for (a) the occlusal surface of the first permanent molar teeth and for (b) the maxillary and mandibular anterior teeth was lower in 1948 than in 1946. The number of first permanent molar occlusal surfaces free from occlusal surface pit and fissure caries, or fillings, was greater for all age groups in 1948 except the 6-year-old group (Tables IV and V).

The permanent tooth surfaces with precarious lesions indicated a drop for 1948 for all three age groups. An anatomic pit, fissure, or groove, is designated as a precarious lesion when there is a questionable etching along the peripheral border or the questionable demonstration of soft material at the base of the pit, fissure, or groove, and yet no frank caries present (Table VI).

TABLE III

DECAYED AND MISSING (EXTRACTED DUE TO CARIES) AND FILLED PERMANENT TEETH. RATE PER HUNDRED CHILDREN

	AGE (YRS.)				PROBABILITY OF DIFFERENCE DUE TO CHANCE*
	6	7	8	6-7-8	
Prefluoride, 1946					
Exams.	461	759	771	1991	
R.P.H.	46.85	153.49	249.93	166.14	
					0.0000
Postfluoride, 1948					
Exams.	756	838	440	2034	
R.P.H.	23.54	103.58	194.09	93.41	

*The probability of this difference being due to chance is 0.0000. Any probability less than 0.05 is conventionally considered significant.

TABLE IV

CARIES EXPERIENCE OF THE OCCLUSAL SURFACE OF THE FIRST PERMANENT MOLAR TEETH. PREFLUORIDE EXAMINATIONS AND 12 TO 22 MONTHS' POSTFLUORIDATION OF THE STUDY AREA WATER SUPPLY

	AGE (YRS.)				PROBABILITY OF DIFFERENCE DUE TO CHANCE*
	6	7	8	6-7-8	
Occlusal Surfaces with Pit and Fissure Caries or Fillings. Rate per Hundred Children					
Prefluoride, 1946					
Exams.	461	759	771	1991	
R.P.H.	40.13	130.03	206.35	138.77	
					0.0000
Postfluoride, 1948					
Exams.	756	838	440	2034	
R.P.H.	19.97	77.32	145.00	70.65	
First Permanent Molars Free From Occlusal Surface Pit and Fissure Caries or Fillings					
Prefluoride, 1946					
R.P.H.	148.37	200.26	180.28	180.51	
					0.0000
Postfluoride, 1948					
R.P.H.	136.37	259.18	242.05	209.83	

*The probability of this difference being due to chance is 0.0000 for the first permanent molars with and without occlusal surface caries or fillings. Any probability less than 0.05 is conventionally considered significant.

TABLE V

DECAYED AND FILLED MAXILLARY AND MANDIBULAR ANTERIOR PERMANENT TEETH. RATE PER HUNDRED CHILDREN

	AGE (YRS.)				PROBABILITY OF DIFFERENCE DUE TO CHANCE*
	6	7	8	6-7-8	
Maxillary Anterior Permanent Teeth					
Prefluoride, 1946					
Exams.	461	759	771	1991	
R.P.H.	0.65	3.03	8.69	4.67	
					0.0000
Postfluoride, 1948					
Exams.	756	838	440	2034	
R.P.H.	0.0	1.67	5.00	1.77	
Mandibular Anterior Permanent Teeth					
Prefluoride, 1946					
R.P.H.	0.86	3.82	5.19	3.66	
					0.004
Postfluoride, 1948					
R.P.H.	0.13	1.37	2.72	1.57	

*The probability of this difference being due to chance is 0.0000 for the maxillary anterior permanent teeth and 0.004 for the mandibular anterior permanent teeth. Any probability less than 0.05 is conventionally considered significant.

TABLE VI

PERMANENT TOOTH SURFACES WITH PRECARIOUS LESIONS (DEEP PIT AND FISSURES)* RATE PER HUNDRED CHILDREN

	AGE (YRS.)				PROBABILITY OF DIFFERENCE DUE TO CHANCE†
	6	7	8	6-7-8	
Prefluoride, 1946					
Exams.	461	759	771	1991	
R.P.H.	50.32	96.83	107.65	90.05	
					0.0000
Postfluoride, 1948					
Exams.	756	838	440	2034	
R.P.H.	19.70	63.00	68.63	48.13	

*An anatomic pit, fissure, or groove is designated as a precarious lesion when there is questionable etching along the peripheral border or the questionable demonstration of soft material at the base of the pit, fissure, or groove, and yet no frank caries present.

†The probability of this difference being due to chance is 0.0000. Any probability less than 0.05 is conventionally considered significant.

The difference in rates per hundred children for 1946 and 1948 is shown in Table VII. A change in one direction or another in the decayed, extracted, and filled deciduous teeth rate for 1948 was expected. This change happened to be in the form of an increase in the decayed, extracted, and filled rate for 1948. As these teeth were erupted, formed, and subjected to a nonfluoride environment for a considerable period of time, very little beneficial effect could be expected from the fluorided water on these teeth. The decrease in the deciduous dentition immunity rate was too small to be of much value as an indication of a true increase or reduction of caries.

TABLE VII

DIFFERENCE IN RATES PER HUNDRED CHILDREN FOUND IN THE 1946 AND 1948 GROUPS OF 6-, 7-, AND 8-YEAR-OLD CHILDREN

	DIFFERENCES
D.E.F.* deciduous teeth	+32.28
Immunities, deciduous teeth	− 0.29
D.M.F. permanent teeth	−72.73
Occlusal surface of first permanent molar teeth with caries experience	−68.12
First permanent molar teeth free of occlusal surface caries or fillings	+29.32
Maxillary anterior permanent teeth D.F.	− 2.90
Mandibular anterior permanent teeth D.F.	− 2.09
Precarious lesions on permanent tooth surfaces	−41.92

*D.E.F. = Decayed, extracted, and filled teeth.

DISCUSSION

The lowering of some of the rates found here can possibly be attributed to things other than sodium fluoride. The brushing of the teeth immediately after a meal is said to retard tooth decay. However, we know quite definitely that the great majority of youngsters in our study do not brush their teeth after the noon meal. The use of the ammoniated tooth powders may have a direct effect. It is even possible, though not probable, that the use of readily fermentable substances, commonly used by children in Evanston, is becoming unpopular.

It is to be expected that the rate of caries in all teeth varies from year to year due to chance. A significant reduction of caries prevalence can therefore be assumed to exist only when the statistical analysis of the data provides almost absolute certainty that the observed differences are not due to chance.

Some of the factors taken into consideration in our evaluations to help to insure valid and reliable findings are: (1) the families using bottled water for cooking and drinking; (2) families maintaining their own water supplies; and (3) cases in which sodium fluoride has been applied topically.

As it is now generally known in Evanston, the study area, that as fluorine is present in the drinking water, the added treatment of topical application of fluorine, except in certain cases, is thought not to be necessary. By means of questionnaires to parents, questioning the child at the chair, and with circulars to Evanston dentists, we feel we are keeping accurate account of the use of sodium fluoride topically.

In 1949 the American Water Works Association gave official acceptance to the practice of water fluoridation for the purpose of reducing tooth decay.[4] This approval by the American Water Works Association may be expected to have a great influence on the development of this practice in the future.

TABLE VIII

COMMUNITIES WITH ARTIFICALLY FLUORIDED WATER SUPPLIES, JANUARY, 1950

STATES	TEST OF DENTAL CARIES-FLUORINE RELATIONSHIP	TOTAL
1. Florida		1
2. Idaho		1
3. Illinois	1	1
4. Kansas	1	1
5. Michigan	1	3
6. New York	1	1
7. North Carolina		1
8. Texas	1	2
9. Wisconsin	3	11
	8	22

SUMMARY

The examinations in the postfluoride period show an increase in the caries rate for the deciduous teeth of the 6- and 8-year-old groups.

The children examined during 1948 showed an over-all lowering in the decayed, missing, and filled rate for all permanent teeth, as well as a reduction in the caries experience of the occlusal surface of the first permanent molars. Accordingly, there was a rise in the number of permanent molars free from occlusal surface caries or fillings, except for the 6-year-old age group. The maxillary and mandibular anterior teeth showed a lowered caries rate for all three ages. There were less precarious lesions-deep pit and fissures in the 1948 examinations.

Statistical analysis of the data indicates a marked reduction of caries prevalence in the permanent teeth of the 6-, 7-, and 8-year-old school children. Comparison of the caries rate in deciduous teeth for the same children, on the other

hand, does not indicate any trend. From the above data the effect of sodium fluoride may be assumed to be greater on the permanent teeth of these children than on their deciduous teeth. The average permanent tooth of these children has been exposed to sodium fluoride for almost its entire posteruptive life. The average deciduous tooth, on the other hand, was present in the child's mouth a considerable time before it was exposed to sodium fluoride.

We feel that while the data indicate some trend that they are far from conclusive. It cannot be assumed at this early time, and cannot be attributed to chance, that the changes that have taken place are necessarily due to the addition of sodium fluoride.

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VARIABILITY IN DENTAL CARIES EXPERIENCE AND ITS IMPLICATION UPON SAMPLE SIZE

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THE advent of possible methods to reduce the extent of dental caries in large groups of people has given considerable importance to the accurate determination of dental caries experience in such groups and the evaluation of differences observed between groups. Counts of decayed, missing, and filled (DMF) teeth are constantly reported in the literature as average values without statistical evaluation. Comparisons of these averages commonly result in misleading statements that the prevalence or incidence of dental caries has been changed by a named percentage as a result of the treatment that was used. Interpretation of the public health importance of such measures as topically applied sodium fluoride treatment depends upon the validity with which collection of the data and statistical comparisons of the results are made.

Good statistical procedure aims to eliminate errors as far as possible and measure those errors which cannot be eliminated. The purpose of this report is to evaluate certain common sources of error in the assembly of DMF counts and suggest methods by which future studies may be designed so as to minimize these errors.

The first job in the elimination of errors lies in the field of common accuracy rather than mathematics. Gross bias must be removed both from the samples used and from the measurement technic. Bias can be thought of as a factor operating to throw the average off in one direction only and is to be differentiated from those random errors or variations which tend to equalize either side of the average. Interpretation as to what actually constitutes a carious lesion must be uniform. The operator's examining error may easily exceed any variability within the sample, especially where many operators are used and a difference in approach exists between operators. Table I will illustrate this point.

TABLE I

DMF PERMANENT TEETH PER PERSON AMONG CHILDREN 12 TO 14 YEARS OF AGE, REPORTED FROM 5 CITIES, BY QUESTIONNAIRE FROM PRIVATE DENTISTS IN 1933-1934* AND BY DEAN, ARNOLD, AND ELVOVE IN 1942†

	1933-1934	1942	DIFFERENCE, PER CENT
Pueblo, Colo.	1.94	4.12	112
Colorado Springs, Colo.	1.65	2.46	49
Elkhart, Ind.	2.20	8.23	274
Michigan City, Ind.	5.25	10.37	98
Lima, Ohio	2.80	6.52	133

*Messner, C. T., et. al. Dental Survey of School Children, ages 6-14 made in 1933-1934 in 26 states, U. S. Public Health Bull. 226, 1936.

†Dean, H. T., Arnold, F. A., Jr., and Elvove, E. Domestic Water and Dental Caries. V., Pub. Health Rep. 57: 1155, 1942.

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The examination data in the questionnaire survey in 1933-1934 came from a great variety of private dentists, school dentists, and in some cases public health nurses, many of them perhaps indifferent examiners and all confronted by patients who were not anxious to be told they had cavities. In contrast, Dean's material was assembled by a trained team of special examiners not responsible for adapting their findings to the treatment desires of the patient. Each survey may be valid for comparisons within itself, but differences between similar values in the two surveys range from 49 to 274 per cent. The brief period of time between the surveys could hardly account for such irregular and often wide differences. Variability within the samples has probably had only a minor effect on average values, for all samples are large. Operator's examining errors of this type within one study should be avoided. Comparisons between two or more studies where method may vary as widely as it does here should not be attempted except to demonstrate the rigidity with which observations must be controlled. In any research paper, the investigator should present a clearly defined description of how the examination was conducted and what criteria for diagnosis were used.

There comes a time in the design of an experiment when bias and examining error have been reduced to a minimum, perhaps to zero, and the errors remaining are inherent in the sample itself: errors due to the diverse heredity, environment, and previous medical history found even in the most carefully selected groups of human beings. Such errors are random in nature and usually tend to even out if the sample studied is large enough. They scatter fairly evenly about the average or mean value for the sample.

A statistician wishing to test the significance of a difference between two such mean values must first determine the reliability of each mean. He has two chief criteria: the width of scatter of individual scores about the mean and the number of individuals measured. The first of these criteria is expressed in a term called the "standard deviation of the observations" usually assigned the symbol σobs and found by the following formula:

$$\sigma_{obs} = \sqrt{\frac{\text{The sum of the squares of all deviations from the mean}}{\text{The number of observations composing the mean}}}$$

Table II deals with a hypothetical example of standard deviation and should make clear why statisticians value this term as an exceedingly descriptive one.

TABLE II

DMF TEETH PER PERSON IN TWO HYPOTHETICAL SAMPLES OF 5 CASES EACH

FIRST SAMPLE			SECOND SAMPLE		
DMF TEETH	DEVIATION*	(DEVIATION)^2	DMF TEETH	DEVIATION*	(DEVIATION)^2
3	−4	16	5	−2	4
5	−2	4	6	−1	1
7	0	0	7	0	0
9	2	4	8	1	1
11	4	16	9	2	4
$5\overline{)35}$	0	$5\overline{)40}$	$5\overline{)35}$	0	$5\overline{)10}$
$\overline{m} = 7$		$\sigma^2_{obs} = 8.0$	$\overline{m} = 7$		$\sigma^2_{obs} = 2.0$
		$\sigma_{obs} = 2.8$			$\sigma_{obs} = 1.4$

*The deviation of the individual count from the average or mean ($\overline{m}$) of 7.

Both samples in Table II are obviously alike in that they have a mean of 7 DMF teeth per person. They are unlike in the way individual cases scatter around this mean. The first sample has twice the scatter of the second sample. In each sample the standard deviation is that of the original observations. Even if each sample contained 10 or any other number of cases for each score group instead of one, the standard deviation of the observations would be the same provided that the cases were equally distributed among the score groups. The standard deviation, therefore, measures a fundamental characteristic of the sample regardless of its size. It tells you whether you are dealing with a "rifle" or a "shotgun." Mathematical formulas are available allowing simpler computation than would be involved by handling large complex samples in the form just shown. They are:

For ungrouped data:

$$\sigma_{obs} = \sqrt{\frac{\Sigma x^2}{n} - \bar{m}^2}$$

For grouped data:

$$\sigma_{obs} = \sqrt{\frac{\Sigma f x^2}{n} - \bar{m}^2}$$

Σx^2 means "the sum of the squares of the (DMF) scores themselves." f means "frequency," where more than one example of a given score occurs and data has been grouped. n means the number of cases in the sample.

The second criterion, sample size, is brought to bear upon the standard deviation by dividing that figure by the square root of the number of cases. Sound reasons can be shown for this procedure. The result is the familiar correction printed after the mean value with a plus or minus sign, thus: 7 ± 1.25. The correction of $\pm$ 1.25 is the "standard deviation of the mean" of the first sample and is found by dividing 2.8, the standard deviation of the observations, by the square root of 5, the number of cases in the sample. In contrast, the standard deviation of the mean of the second sample is 7 ± 0.63. It is obvious that the larger the sample becomes the smaller is the correction to be applied to the mean and the more reliable the mean becomes for statistical purposes. I will not describe in detail how standard deviations of means are used in carrying through a "statistical significance test," but the procedure is an easy one[1] and gives the probability that a given difference between specific means could have occurred by chance. The answer depends fully as much on the size and variability of the means as it does upon the actual difference between them. The chief formulas involved are:

$$\sigma_\Delta = \sigma_{obs} \sqrt{\frac{1}{n_1} + \frac{1}{n_2}} \quad \text{and C.R.} = \frac{\Delta}{\sigma_\Delta}$$

Δ refers to the difference between means. n_1 and n_2 are the numbers of cases in each sample respectively. C. R. is the critical ratio. Ratios of 2.0 and 3.0 are border line, the lower figure implying about a 5 per cent probability of chance occurrence, the upper figure implying about 0.3 per cent probability.

When we apply these methods to dental caries findings it is obvious that the accuracy of comparisons between experimental and control groups will depend a great deal upon the standard deviations of the observations. One notices at once that human DMF counts vary exceedingly widely. It is quite common for even a fairly homogeneous group of young adults to show the extreme range from 0 to 32 DMF teeth and for the standard deviation of the observations about the mean to be well above 4 teeth. It is almost impossible, in fact, to find a sample above age 20 where the standard deviation is less than 4.0. Heterogenous samples will vary more widely. The main purpose of this report is to illustrate the variability of DMF findings as a caution to those who have the task of interpreting dental research reports.

Table III represents the work of 5 Navy dental officers working under hurried conditions. The completeness of the data is open to doubt but there is no reason to believe that variability between individuals was exaggerated. The midshipmen under study were as homogenous a group as one could hope to find. They represented careful physical and mental selection. They were centered on age 21, five-eighths of the group being actually 21 or 22 years old. In spite of variations within each regional sample, the means differ by an amount which, when tested, proves highly significant.

TABLE III

FREQUENCY OF DMF TEETH, THIRD MOLARS EXCLUDED, AMONG 275 MIDSHIPMEN, AGE APPROXIMATELY 21, SELECTED BECAUSE OF BIRTH IN ONE OF TWO SELECTED AREAS, U.S.N.R.-M.S., NEW YORK, 1944

DMF TEETH	FREQUENCY OF CASES	
	MIDDLE ATLANTIC STATES	SOUTHWESTERN STATES
0-1	2	9
2-3	5	6
4-5	12	10
6-7	24	12
8-9	36	8
10-11	42	6
12-13	22	4
14-15	29	5
16-17	20	5
18-19	10	0
20-21	4	0
22-23	3	0
24-25	1	0
	210	65
Mean:	11.21 teeth	7.36 teeth
Standard deviation of observations:	4.58 teeth	4.81 teeth

Difference between means: 3.85 teeth
Standard deviation of difference: 0.702 teeth
Critical ratio: $\frac{3.850}{0.702}$ or 5.49. A critical ratio as large as this implies that such a difference could have occurred by chance less than once in a million tests with samples of the sizes stated

Table IV describes a study group assembled from the Greater Boston area by combining the findings from 8 public schools, 4 schools into Group X, and 4 into Group Y. Two recent dental graduates did all the examining, their findings being compared for general consistency with examinations of the same

children made the year before. The school samples were arranged to form groups that had mean DMF values as closely similar as possible at the different ages, but samples were taken in their entirety and not subdivided between Groups X and Y.

TABLE IV

DMF PERMANENT TEETH AT DIFFERENT AGES AMONG PARALLEL GROUPS OF BOSTON SCHOOL CHILDREN SELECTED FOR EXPERIMENTAL PURPOSES TO GIVE CLOSELY SIMILAR MEAN VALUES AT EACH AGE*

	GROUP X			GROUP Y		
		DMF TEETH			DMF TEETH	
AGE	NO. CASES	MEAN	σobs	NO. CASES	MEAN	σobs
7	113	2.02	1.62	102	1.54	1.77
8	224	2.55	1.77	224	2.56	1.84
9	204	3.41	2.21	204	3.13	2.09
10	221	4.39	2.72	207	4.24	2.49
11	202	5.42	3.26	205	5.40	3.16
12	160	6.50	3.55	157	7.16	4.11
13	43	8.26	4.76	51	7.98	3.93
All ages	1167			1150		

*Unpublished data from the Dental Division of the Massachusetts Department of Public Health, W. Wellock, 1948.

It is interesting to apply a significance test to the largest difference between means in this table: the difference between 2.02 and 1.54 DMF teeth at the age of 7. Although one mean is 24 per cent less than the other, the difference is only 0.48 ± 0.23 teeth. The critical ratio is 2.1 and of borderline significance since differences of this magnitude might be expected to occur by chance almost 5 per cent of the time.

Table V shows a long-range study group, perhaps more diverse physically than either of the foregoing groups but selected chiefly for a willingness to return year after year for physical and dental examination. One operator from the dental staff of the Children's Hospital was involved. He may be presumed to have been careful, since time and equipment were adequate.

TABLE V

DMF PERMANENT TEETH AMONG 119 CHILDREN AT DIFFERENT AGES. HARVARD SCHOOL OF PUBLIC HEALTH LONG-TERM CHILD STUDY GROUP, BOSTON, 1948

		DMF TEETH		
AGE	NO. CASES	MEAN	RANGE*	σobs
10	10	3.90	0-5	0.95
11	15	5.16	0-11	3.00
12	20	8.40	2-23	4.54
13	22	8.23	2-19	4.41
14	24	9.59	4-23	4.38
15	22	11.77	6-25	4.67
16	6	11.16	6-17	3.64†
	119			

*Extreme low and high scores.
†This value is open to question because of the small size of the sample.

The striking thing about all 3 samples is the way that, regardless of mean DMF value, all standard deviations of the observations tend toward values between 4 and 5 teeth per person when all the permanent teeth have erupted.

This is a high standard deviation and reflects a wide range between extreme scores, as Tables III and V illustrate. All samples were carefully chosen: the midshipmen for physical and mental excellence, the Boston school children for similar mean DMF values, the Harvard group for cooperation over the years. Two samples were divided into one year age groups and the third (midshipmen) was much more closely focussed on one age than would be true in the 5-year age groups so often used for dental caries studies. Greater homogeneity in samples and smaller variability in DMF counts will be hard to attain. One sees the inherent difficulty attending all studies on human beings and can understand better why research men turn to experimental animals in order to control such major variables as heredity and nutrition. Where human material must be used, statistical significance tests should be performed and reported. If this is not done, frequency distributions of the DMF or other scores should be published so that the reader may perform his own tests.

What is the implication of this high variability upon the design of experiments to test methods of caries control? Fortunately, the apparent tendency for standard deviations to settle between 4.0 and 5.0 in young adults gives us a hint. The formula for statistical significance test given earlier can be reversed and solved for the number of cases necessary in each sample, if fixed values are assumed for the difference between means and for the standard deviation. The formula thus becomes:

$$n = \frac{18\ \sigma^2_{obs}}{\Delta^2}$$

The constant, 18, implies a critical ratio of 3.0 giving a less than 1 per cent chance that the difference Δ between means will have occurred by chance. A less conservative approach would be with a critical ratio of 2.0, giving 5 per cent probability of a chance difference. The constant would then be 8. n is the number of cases in *each* sample, experimental or control, and these samples are presumed to be equal in size.

TABLE VI

MINIMUM SAMPLE SIZES, EXPERIMENTAL OR CONTROL, NECESSARY TO REDUCE CHANCE OCCURRENCE OF A STATED DIFFERENCE BETWEEN MEANS TO BELOW 1 PER CENT,* IF EXPERIMENTAL AND CONTROL GROUPS ARE EQUAL IN SIZE

EXPECTED DIFFERENCE BETWEEN MEANS	NUMBER OF CASES, EACH SAMPLE, WHEN:		
	σobs = 3.0	σobs = 4.0	σobs = 5.0
1.0	162	288	450
2.0	41	72	113
3.0	18	32	50
4.0	11	18	29
5.0	7	12	18

*A critical ratio of 3.0 is assumed, giving a probability of 0.3 per cent that the difference between means might have occurred by chance.

A convenient table for size of sample (Table VI) can be constructed from this formula. No investigator will know in advance the exact means and standard deviations that his experiment will produce; otherwise, he would not need to perform it. The general range of mean and standard deviation values are often predictable, however, and permit some rough estimate of the sample sizes needed. The inference is obvious that where small differences in mean

values are expected, very large samples must be used. By making the samples a great deal larger than the minimum, additional accuracy can be obtained and unforeseen errors allowed for, provided of course that in making the increase in numbers one does not unduly impair the quality of the sample or quality of the examination method.

Knutson and his colleagues in the U. S. Public Health Service group studying topical fluoride application have encountered wide variability between individuals in their samples. Only one of their published reports contains data on individuals.[2] Here, although the final result shows the 40-odd per cent benefit from treatment so well known in all their papers, some individuals are shown to have developed more carious lesions on the treated side than on the untreated. This is a perfectly probable phenomenon. Only one lesion on the treated side is needed to place an individual in the category named, and it is well known by now that sodium fluoride protects certain teeth less effectively than others. Knutson and Armstrong, however, in subsequent reports[3] abandon a tabulation of results by individual cases. They solve the problem of statistical analysis in a different way from the one outlined above, expressing their results in terms of teeth, not persons. Thus there is no mean for the individual "case": a tooth is either listed as attacked (D,M, or F) or not attacked. When they add up DMF totals, a series of ratios or proportions accumulate between the attacked and unattacked teeth. The differences between such proportions are easier to test than differences between means: a different technic is used not requiring frequency distributions.[4] The standard deviation of the difference between proportions is found by the formula:

$$\sigma\triangle = \sqrt{\frac{p_1(100-p_1)}{n_1} + \frac{p_2(100-p_2)}{n_2}}$$

where p_1 and p_2 are the average proportions of attacked teeth (expressed as percentages) found in the experimental and control groups, respectively. n_1 and n_2 are the measures of group size, in this instance number of teeth, not persons. A critical ratio is then found and interpreted by the method already described.

A typical example for testing is found in Knutson and Armstrong's second year study[5] where they report 97 carious teeth in 1944 from a group of 929 erupted but unattacked upper left teeth treated with sodium fluoride in 1942. The untreated upper right teeth showed 173 carious in 1944 out of 940 erupted but unattacked in 1942. The proportion of new caries on the experimental side is 10.4 per cent; on the control side, 18.4 per cent. The first figure is about 56.5 per cent of the second, giving about 43.5 per cent reduction in dental caries. A significance test may be done as follows:

$$\text{C.R.} = \frac{18.4-10.4}{\sqrt{\frac{18.4 \times 81.5}{940} + \frac{10.4 \times 89.6}{929}}} = \frac{8.0}{1.6} = 5.0$$

As with the Columbia midshipmen, this result implies a probability of the general magnitude of one in one million that such a difference would have occurred by chance.

The statistical analysis of Knutson's work is most convincing and it applies to large groups of cases, yet his particular method of presentation leaves unanswered several questions we must face sooner or later. The probability of benefit to the individual patient and the best ways for further separation of benefited patients into hereditary and environmental groupings not heretofore studied are among the most important of these questions. Moreover the true variability of the sample is discarded in favor of an artificial variability which is all one can find when data is reported on a proportion basis. It is to be hoped that important studies by Knutson and his co-workers will, in the future, be presented in terms of persons as well as in terms of teeth.

SUMMARY

1. Interpretative and other examining errors in DMF studies may be large, easily exceeding a 100 per cent difference between samples. In any research paper the investigator should present a clearly defined description of how the examination was conducted and what criteria of diagnosis were used. From these data the statistician must make common-sense evaluation of the accuracy of examinations prior to statistical testing of results.

2. Illustrations of actual data indicate that the standard deviations of observations about the means (averages) in DMF studies are large even where examining errors are reduced to a minimum. Standard deviations between 4 and 5 DMF teeth per person seem usual in currently available study groups in this country when the individuals are over the age of 12 years.

3. These two sources of variability imply that human DMF studies should be subjected to close scrutiny as to the validity of the data and statistical significance tests applied and reported wherever possible. Mere statements that "caries was reduced by x per cent" are not sufficient.

4. Where some estimate of the possible caries differences and standard deviations can be made in advance, experiments can be designed to have samples of sufficient size to meet the ordinary statistical tests. A formula and table are presented for the calculation of minimum sample size.

5. Presentation of data in terms of teeth rather than individuals obscures important findings relative to individual reactions, although it simplifies statistical analysis.

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INTRAVITAL UPTAKE OF FLUORIDES BY THE TEETH UNDER VARIOUS CONDITIONS

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FROM the report of Knutson[1] it is evident that the caries-reducing effect of the topical fluoride treatment of teeth depends on several factors including the number of applications, whether prophylaxis was given prior to treatment, and the age of the individual treated. The mode of action of fluorides is not clearly understood. On the one hand, they increase the resistance of enamel against acid solvents and, on the other hand, they interfere with the degradation of carbohydrates to acid.[2] Whether or not the protective effect of topical treatments depends upon the amount of fluoride taken up by the teeth has not been demonstrated, though clinical experience seems to support the existence of some quantitative connection, a certain optimum saturation; four treatments, the first one preceded by prophylaxis, produce the maximum effect.

It seems to us worth while to estimate the uptake of fluorides by the teeth in vivo from a topically applied sodium fluoride solution under different conditions, comparable to those of clinical experiments. Behavior of teeth intravitally differs in some fundamental respects from the reactions of pulverized enamel with fluorides in vitro. This is demonstrated cogently by the discrepancies between laboratory tests with pulverized enamel treated by a lead fluoride[3] or by an acidulated sodium fluoride solution[4] on the one hand, and experience in laboratory animals[5] and in man[6, 7] on the other hand.

METHOD

Uptake of fluorine by noncarious pairs of lower premolars was examined in vivo. One premolar on each side was separated from the oral cavity by a thin rubber balloon. This was fastened on the tooth like a rubber dam by aid of a clamp. The rubber sac fits closely to the neck of the tooth and is practically impermeable to fluids. Five to twenty milliliters of a sodium fluoride solution were placed into each sac and the open end of the sac was closed tightly by a screw clamp. Patients were asked not to close the mouth and saliva was removed by an ejector (Fig. 1). Involuntary movements of the mandible and cheeks resulted in some agitation of the fluid occurring within the sac. After the relapse of 5 to 12 minutes, the patients were instructed to bend the head as far forward as possible. The fluid within the sac then flowed to the screw-clamped end. The sacs were cut near the teeth, most of the fluid having been collected in the dependent part (Fig. 2). This was then emptied into a small bottle. Some fluid remains in contact with the tooth and in the remnant of the sac. This is removed by the saliva ejector and is not used in the fluorine estimations.

Uptake of fluorine is computed by subtracting the concentration of F ions at the end of each individual experiment from the original concentration. This was computed first for 1 ml. of the solution and then multiplied by the number

of milliliters used. This method has been successfully used in our former experiment[8] in estimating the fluoride saturation rate of pulverized enamel. It offers the advantage that it is not necessary to collect the total amount of fluid used at the end of each experiment.

Fig. 1.

Fig. 2.

Fig. 1.—The rubber sacs are filled with 20 and 10 ml. solution, respectively, and held in upright position by a weight hanging down behind the head.

Fig. 2.—The head is bent forward. Fluid is collected in the dependent part of the sacs, near the clamps. On the right side the sac has just been cut.

The original concentrations of sodium fluoride solution used varied between 1.0 and 2.0 mg. in 100 ml. This low concentration was found the most advantageous for accurate estimation of small fluorine quantities. In former experiments[8] it proved to be sufficient to cause complete saturation of pulverized enamel within due time. In most experiments a neutral solution was used. In one series, however, an acidulated low concentration solution produced by dissolving NaF in a lactate buffer of approximately pH 4.0 was used. In fluorine estimation the von Fellenberg[9] modification of the Willard-Winter method was used. Experiments were carried out on inclinic and outclinic patients consenting to experimentation, and, chiefly, on inhabitants of "Boys' Town" at Debrecen, during May to August, 1949.

RESULTS

For comparing the influence of different items upon the uptake of fluorides by the intact tooth surface in vivo the most proper method appeared to be by comparison of the uptake by bilateral pairs of teeth. The variations in uptake by bilateral pairs under identical conditions were first determined.

Five experiments were carried out on boys, 15 years of age. The results are summarized in Table I. In three instances there was practically no difference at all, and in two instances there was some difference (20 to 21 per cent). The difference between bilateral pairs averages somewhat less than 10 per cent.

Subsequently, in 9 persons the uptake was estimated after one lower premolar had received "prophylaxis" prior to fluorinization, and the other had not. In all experiments a considerable difference of the uptake was stated. The smallest difference was 50 per cent lower uptake by the tooth without prophylaxis but in several instances variations up to 100 per cent occurred. The average difference was to approximately 55 per cent. Apparently, the longer the exposure to the fluoride solution the greater difference between the pairs.

TABLE I

UPTAKE OF FLUORINE UNDER IDENTICAL CONDITIONS BY BILATERAL PAIRS OF LOWER PREMOLARS

NAME	DMF* COUNT	AGE	PRO-PHY-LAXIS	TOOTH	F IN GAMMA (TOPICALLY APPLIED)	ML. SOLUTION (TOPICALLY APPLIED)	DURATION OF EXPOSURE IN MINUTES	UPTAKE OF F IN GAMMA BY RIGHT LOWER PREMOLAR	UPTAKE OF F IN GAMMA BY LEFT LOWER PREMOLAR
Z. J.	3	15	no	4	100	10	7	52.2	53.0
U. L.	5	15	no	4	100	10	10	24.1	25.5
Z. M.	14	15	no	5	100	10	15	25.0	30.0
S. G.	10	15	yes	5	100	10	10	19.2	18.2
U. J.	3	15	yes	4	200	20	5	12.4	16.0
Average								26.6	28.5

*Decayed, missing, and filled teeth.

Several estimations were carried out to determine the influence of varying quantities of fluoride upon the uptake. Results are summarized in Table III. In the first 5 experiments the double quantity of an identical solution was used on the left side. In two individuals the lower left second premolars were missing (probably not yet erupted). In these boys apparently there was contact between fluoride solution and greater tooth surface. Thus, on the left side, the uptake was considerably greater. In the subsequent 3 experiments, where identical conditions prevailed bilaterally, there were no appreciable differences. This indicates that within 5 to 15 minutes the fluorides from 10 ml. solution, containing 100 gamma fluoride ions, are not consumed to a degree that a further uptake could be interfered with.

TABLE II

THE EFFECT OF PROPHYLAXIS PRIOR TO TOPICAL TREATMENT UPON THE UPTAKE OF FLUORINE

NAME	DMF COUNT	AGE	PRO-PHY-LAXIS	TOOTH	F IN GAMMA (TOPICALLY APPLIED)	ML. SOLUTION (TOPICALLY APPLIED)	DURATION OF EXPOSURE IN MINUTES	UPTAKE OF F IN GAMMA ON THE SIDE WITH PROPHYLAXIS	UPTAKE OF F IN GAMMA ON THE SIDE WITHOUT PROPHYLAXIS
F. P.	x	18	right	5	100	10	20	37.4	19.2
T. I.	x	17	right	4	100	10	20	19.5	10.0
M. P.	x	16	right	4	100	10	20	31.8	16.0
Z. L.	x	22	right	4	100	10	20	8.2	4.0
P. M.	x	21	right	4	100	10	20	3.7	1.9
T. A.	0	15	right	4	200	10	15	141.0	102.0
I. T.	8	16	left	4	100	10	17	38.3	23.3
H. S.	5	16	left	4	100	10	15	65.2	47.4
S. I.	3	13	left	4	100	10	20	31.0	16.5
Average								41.8	26.6

x: DMF counts are not recorded.

Seven boys were utilized to ascertain whether increasing the fluoride concentration exerts any influence upon the uptake. With the exception of D. J.

TABLE III

INFLUENCE UPON FLUORINE UPTAKE OF AMOUNT OF FLUORIDE PRESENT IN TOPICALLY APPLIED SOLUTION

NAME	DMF COUNT	AGE	PROPHY-LAXIS	TOOTH	DURATION OF EXPOSURE IN MINUTES	RIGHT SIDE			LEFT SIDE		
						ML. SOLUTION APPLIED	F IN GAMMA APPLIED	UPTAKE OF F IN GAMMA	ML. SOLUTION APPLIED	F IN GAMMA APPLIED	UPTAKE OF F IN GAMMA
K. E.*	0	15	yes	4	8	10	100	15.0	20	200	30.8
D. L.*	0	13	yes	4	15	10	100	18.0	20	200	35.4
S. K.	3	14	yes	4	15	10	100	42.5	20	200	45.0
K. K.	7	15	yes	4	15	10	100	25.0	20	200	24.0
O. I.	6	15	yes	4	5	10	100	14.5	20	200	16.0
D. J.†	3	15	yes	4	10	10	100	12.0	10	200	10.0
D. T.	4	16	yes	4	15	10	100	44.0	10	200	45.0
S. J.	3	14	yes	4	10	10	100	65.0	10	200	84.0
N. B.	5	16	yes	4	5	10	100	39.5	10	200	42.0
C. Z.	3	15	yes	4	15	10	100	30.0	10	200	35.0
L. B.	6	15	yes	4	5	10	100	70.0	10	200	90.0
M. R.	11	15	yes	4	5	10	200	30.0	10	100	31.0
B. I.	0	14	yes	5	15	20	200	6.0	10	200	14.0
H. J.	0	15	yes	4	15	5	100	9.0	10	100	3.0

*The adjacent second premolar was not erupted.
†Rachitic hypoplastic teeth.

with rachitic hypoplastic teeth, teeth exposed to the action of a doubly concentrated solution showed some increase in uptake. The maximal difference was approximately 30 per cent, but the differences were mostly within the limits found in comparing uptake of bilateral pairs under identical conditions. The concentration of fluoride seemingly was not important if a sufficient quantity of the solution was used. Nevertheless, if a small amount of fluid is placed in the sac, the concentration of the fluoride may play a role. The results of the two last experiments indicate a beneficial influence of the higher fluoride concentration.

To find the influence of the duration of exposure two procedures were used. In one series of experiments bilateral pairs of teeth were simply compared, the one being exposed for 5 minutes less than the other. The longer exposure resulted in an increased uptake. In a second series, 20 ml. NaF solution containing 200 gamma fluoride was placed in each sac. From one sac, at intervals of 5 minutes, 2 ml. of the NaF solution was pipetted away and the total loss of fluorides was computed. On the other side the usual procedure was followed. This procedure gives an opportunity to examine the fluoride saturation rate of teeth in vivo, and, at the same time, a control for subsequent estimations. The differences between the bilateral pair in those four experiments, where the control extended for 20 minutes, were within the limits of variance of Table I. In most instances the uptake within the first 5 minutes was somewhat slower than later, but, after the elapse of 15 minutes, a marked decrease in uptake was observed. This is a clear-cut difference between fluoride uptake by enamel in vivo and by pulverized enamel in vitro. The results indicate that within 15 minutes a nearly complete saturation may be attained.

TABLE IV

INFLUENCE OF TIME OF EXPOSURE UPON UPTAKE OF FLUORINE

NAME	DMF COUNT	AGE	PROPHYLAXIS	F IN GAMMA APPLIED	ML. SOLUTION TOPICALLY	TOOTH	RIGHT SIDE EXPOSURE IN MINUTES	RIGHT SIDE UPTAKE OF F IN GAMMA	LEFT SIDE EXPOSURE IN MINUTES	LEFT SIDE UPTAKE OF F IN GAMMA
S. P.	0	15	no	100	10	5	10	50	15	60
K. J.	12	16	yes	200	20	4	15	36	10	24
V. I.	4	16	yes	200	10	4	10	60	5	10

Fluoride uptake from an acidulated solution was examined. Acidulated solutions are known to exert harmful effects, but, because of interest on this problem, a very few experiments were carried out. The results undoubtedly indicate that the loss of fluorides is promoted by acidulation of the fluoride solution. Based upon our method of estimation, i.e., computing from the loss of fluoride concentration, no statement can be made whether or not the fluorides had been actually taken up by the enamel. It is undoubtedly possible that precipitation of calcium fluoride occurred with calcium salts dissolved from the enamel by the lactic acid buffer, and this may be responsible for the greater loss of fluorides from the solution. With this consideration in mind, prophylaxis was given in all experiments with a water-pumice mixture rather than with a peroxide-pumice mixture.

From the data in Table II it is evident that with increasing age, beyond 20 years, there is a considerable decrease in the quantity of fluorides taken up. In Table II the data from two persons are tabulated, but three further estimations were run. In no instance did the uptake exceed 10 gamma in 20 minutes, after prophylaxis. In persons of 13 to 16 years the uptake within 10 minutes was not less than 10 gamma in any instance, and usually considerably more. In a preliminary report of Adler and Straub[10] the uptake in 5 persons over 20 years of age averaged approximately 6 gamma, in 5 persons between 12 and 18 years of age approximately 45 gamma, within 20 minutes, and after prophylaxis. More extended studies confirmed these results. The individual variations of uptake are too great to permit conclusion as to the expected uptake of fluorides at any given age.

TABLE V

RATE OF FLUORINE UPTAKE FROM 20 ML. NaF SOLUTION CONTAINING 200 GAMMA FLUORINE

NAME	DMF COUNT	AGE	TOOTH	PROPHYLAXIS	UPTAKE OF FLUORINE IN THE PERIOD BETWEEN 0-5 | 5-10 | 10-15 | 15-20 MINUTES OF EXPOSURE IN				TOTAL UPTAKE OF F IN GAMMA	UPTAKE BY CONTROL SIDE IN GAMMA	UPTAKE BY CONTROL SIDE IN MINUTES
V. A.	0	14	4	yes	20.0	20.7	21.0	6.6	68.6	75.0	20
G. A.	0	14	4	yes	24.0	34.2	7.2				
B. E.	2	16	5	yes	16.0	42.0	8.8	3.6	75.4	68.0	20
B. Z.	0	15	4	yes	20.0	43.5		4.8	68.3	64.0	20
E. J.	0	14	4	yes	9.0	56.0	16.0	2.0	73.0	68.0	20
U. J.	3	14	4	yes	12.0	24.0	5.6	5.6	51.2	12.4	5

DISCUSSION

It was intended to determine whether or not there is a relation between some of the facts established by clinical experience and the uptake of fluorides by the teeth, intravitally. It is well known that the caries-reducing effect of the topical fluoride treatment is approximately doubled by a prophylaxis prior to fluorination. Our findings are in agreement with this experience. Prior prophylaxis considerably increased the uptake of fluorides.

TABLE VI

UPTAKE OF FLUORINE FROM NEUTRAL AND FROM ACIDULATED NaF SOLUTION

NAME	DMF COUNT	AGE	TOOTH	PROPHYLAXIS	TIME OF EXPOSURE IN MINUTES	F IN GAMMA APPLIED TOPICALLY	ML. SOLUTION APPLIED TOPICALLY	UPTAKE OF F IN GAMMA FROM THE SOLUTION NEUTRAL	UPTAKE OF F IN GAMMA FROM THE SOLUTION ACIDULATED
C. S.	x	14	4	yes	10	100	10	36.0	71.0
G. A.	0	15	4	yes	10	100	10	53.0	73.5
G. L.	0	13	4	yes	10	100	10	—	45.5

x: DMF count was not recorded.

It has been similarly shown that fewer than four treatments resulted in a less marked caries reduction. Reducing the number of treatments means shortening the time of exposure to fluorides. By shorter exposure, as has been shown by our experiences, the uptake of fluorine is lessened.

Clinical experience affords no data to rely upon concerning the importance of the sodium fluoride solution. Undoubtedly, in all clinical experiments up to

the present, too strong solutions were used. Our experiences indicate that low concentration solutions may cause a sufficient saturation of the enamel surface by fluorides within due time.

In reporting the results of experiments with an acidulated fluoride solution we have pointed out the inadequacy of the test method used by us in examining this peculiar problem. Thus, the increased loss of fluorine from an acidulated solution has no bearing upon increasing resistance of the tooth.

Our estimations seemingly support the view that the caries-protecting effect of the topical fluoride treatment depends on the amount of fluoride taken up by the teeth.

A problem of some interest is whether or not the fluorine uptake of teeth is greater in caries-susceptible persons than in those who are caries immune. If caries-susceptible teeth absorb more fluorides this would suggest that caries susceptibility is due to lack of fluorides in the superficial enamel layers. We tried to correlate our findings from this aspect. According to our judgment, we are unable to find any positive correlation. This is of interest since Hattyasy claimed that the penetration of dyes into the enamel is more marked in caries-susceptible teeth than in those that are caries resistant.[11] Lower premolars, especially the first premolars, are relatively immune to caries. Perhaps this may be responsible for our failure to find any difference similar to that found by Hattyasy. However, it should be remembered that uptake of fluorides and penetration by dyes are fundamentally different processes.

SUMMARY

Fluorine uptake, by noncarious teeth, was estimated under different conditions. Bilateral pairs of teeth show practically equal uptake under identical conditions. Prophylaxis prior to fluoride treatment increased the uptake considerably. From 20 ml. of a solution containing 200 gamma F there is no increased uptake in comparison to 10 ml. of the same solution. Increasing the concentration to 200 gamma in 10 ml. from 100 gamma in 10 ml. causes no increased uptake. Longer exposure results in an increased uptake. Within the first 15 minutes the greatest quantity of F is taken up but uptake is reduced after the elapse of 15 minutes. Acidulated NaF solutions lose more F than neutral ones but this in no way proves that the loss is due to fluorine uptake by the enamel and not to precipitation of calcium fluoride. Individuals over 20 years of age show a considerably lessened uptake in comparison to those below 18 years. There is no distinct connection between caries experience and fluoride uptake.

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ELECTRON MICROSCOPY OF HUMAN DENTIN

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IN A previous publication[1] we have described results obtained through the electron microscopic observation of metal-shadowed collodion replicas of the enamel and dentin of teeth. In this earlier work it was shown that the ends of the dentinal tubules could be reproduced in the replicas, and the amount of detail revealed could be partly controlled by varying the concentration and type of acid used for etching. It was also found that in some instances all of the dentinal fibers were not removed from the collodion during the dissolution processes and that those remaining could be examined on the replica.

The present paper describes further studies of such preparations from dentin. They involve the investigation of replicas of sections from about 500 teeth. The teeth employed have been noncarious molars and premolars that were placed in 3 per cent formalin immediately after extraction and used within a few days thereafter. Cross and longitudinal slabs of dentin were cut and their surfaces polished according to the technics previously described,[1] except that the sectioning was carried out with a newly built machine capable of cutting large numbers of slabs from a single tooth. The surfaces of the slabs were comparatively smooth and required little polishing with metallographic papers.

The method of making the shadowed replicas[1] consisted in etching the polished slabs with acid to delineate the histologic structures. The etched surfaces were dried and covered with a dilute solution of collodion in ether. When the collodion had dried, the sections were floated, collodion side up, in HCl until the enamel and dentin had dissolved, leaving the replicas on the surface of the acid. The replicas were transferred for further cleaning to a pepsin solution and finally washed by flotation on the surfaces of several changes of distilled water. Specimen screens for the electron microscope were placed on the replicas, which were face down on the water, and screens and replicas together were picked up with absorbent paper. Finally, the replicas were shadowed with chromium and examined with an RCA type EMU electron microscope. Approximately 2,000 specimen screens have been studied and 3,000 micrographs taken.

The study of fibrous components of dentin remaining attached to the replicas has proved to be more informative than observation of the replicated ends of dentinal tubules. There are numerous ways of preparing replicas with these attached fibers. If the surfaces of the slabs are etched for 5 to 10 seconds with 0.1N HCl prior to replication, large numbers of fibers are usually obtained; smaller numbers can be obtained by decreasing the acid concentration and etching time, or by using a weaker acid. Experience has shown that if replicas

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are removed from the acid before dissolution of the slabs is complete, as manifested by a slightly cloudy appearance of the replica, then a plentiful residue of fibrous material will usually remain after the pepsin treatment and final rinses. The quantity of fibrous material obtained apparently varies with the thickness of the sections, larger numbers of fibers usually being obtained from the thicker sections. The number of remaining fibers also depends on the pepsin used in the cleaning process and tends to be greater when less active pepsin is employed. These methods for controlling the amount of recoverable fibrous material are difficult to standardize and are often unpredictable in their results, but they have provided many instructive specimens.

Replicas of the ends of dentinal tubules.—It was previously noted[1, 2] that when dentin was etched lightly with dilute acid, e.g., for 5 seconds with 0.4N citric acid, replicas of the ends of the dentinal tubules showed a central fiber and a thin wall-like structure at the periphery. This peripheral structure is of special interest as possibly being the controversial Neumann's sheath. Unfortunately, the evidence which we have been able to accumulate through further study does not yet conclusively demonstrate whether or not this wall-like boundary of the tubules is a sheath. In favor of its being a sheath is the fact that it appears as an entity in itself at the border of the tubule as shown in Figs. 6 and 7 of Scott and Wyckoff's study[1]; when dentin is etched so lightly that structural detail is extremely sparse, it is the only structure made visible (Fig. 1). Against its being a sheath is the fact that it has not been possible by the methods used to isolate a tissue which could be identified as this sheath, though large quantities of the central fibers have been readily obtained. On the other hand, it is conceivable that the wall structure seen on the replicas represents a space into which collodion has flowed, for even when the etch is sufficient to produce great lengths of dentinal fibers the wall seldom is higher than that noted when the etch is much lighter (Fig. 2). In any event, the suggestion sometimes offered[3] that the sheath of Neumann is an optical artifact due to shrinkage of the dentinal fiber does not adequately explain our observations. If it were the result of such a shrinkage phenomenon the narrow peripheral structure would not appear separately at some distance from the dentinal fiber (Fig. 2), but instead would be a wide band extending from the fiber to the outer wall of the tubule.

Dentinal fibers.—The dentinal fibers (Tomes' fibers) are surprisingly resistant to the strong acid and pepsin solutions to which they are exposed. Intact fibers are often found in the normal relationship to the tubule (Fig. 2), or they may be scattered at random on the replica (Fig. 3). In many cases branches have been seen connecting parallel fibers (Fig. 4). Intact fibers are ordinarily opaque under the electron microscope, but large numbers of torn fibers have been seen and these have permitted study of their walls and contents. The outer walls of the fibers appear to be composed of a thin, membranous tissue (Fig. 3). There is a suggestion that additional fibers may be present within each dentinal fiber. Many such bundles of smaller fibers surrounded by torn membranous tissue have been observed (Figs. 5 and 6), often emanating from intact fibers having the size of the optically recognized dentinal fibers.

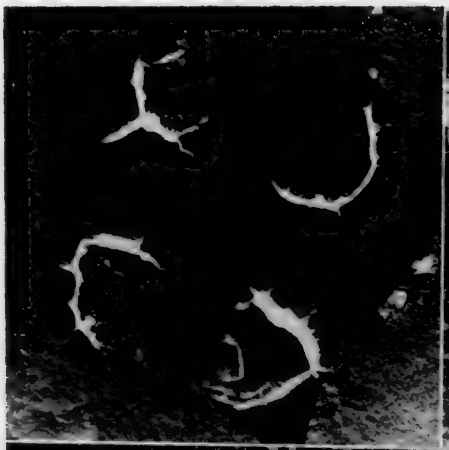

Fig. 1.

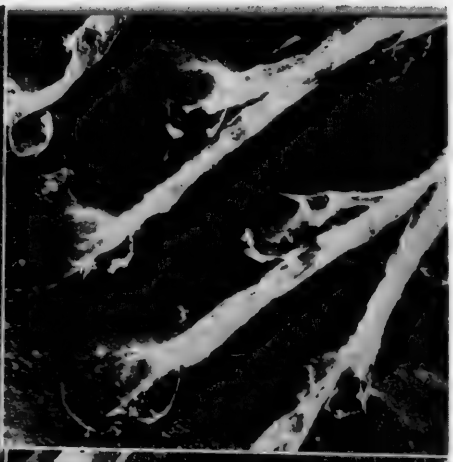

Fig. 2.

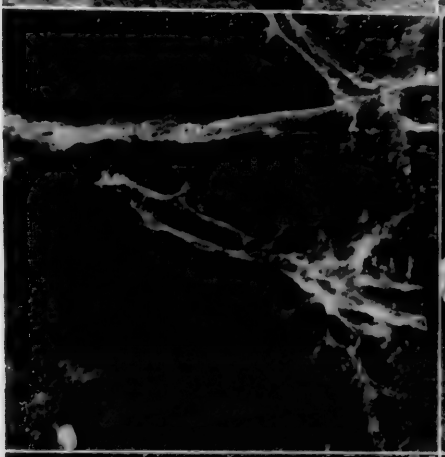

Fig. 3.

Fig. 4.

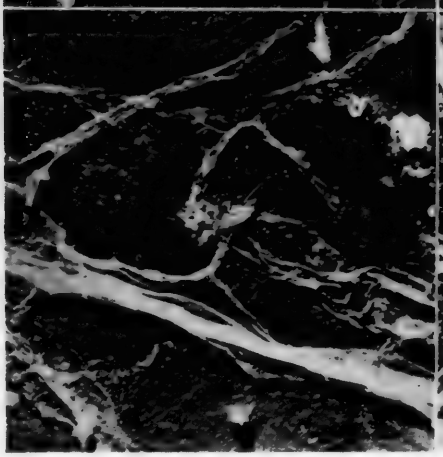

Fig. 5.

Fig. 6.

Fig. 1.—Lightly-etched dentin; etched 5 seconds with 0.1N citric acid. (×5,500.)
Fig. 2.—Dentinal fibers; dentin etched 10 seconds with 0.1N HCl. (×5,350.)
Fig. 3.—Outer wall of a dentinal fiber; dentin etched 3 seconds with 0.1N HCl. (×5,550.)
Fig. 4.—Branched dentinal fibers; dentin etched 10 seconds with 0.1N HCl. (×5,600.)
Fig. 5.—Dentinal fiber torn open; dentin etched 5 seconds with 0.8N citric acid. (×7,350.)
Fig. 6.—Dentinal fiber torn open; dentin etched 5 seconds with 0.8N citric acid. (×7,350.)

Fig. 7.

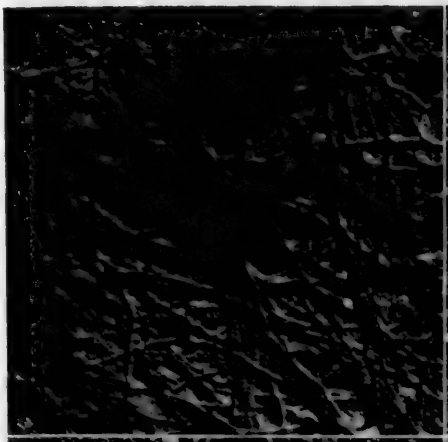

Fig. 8.

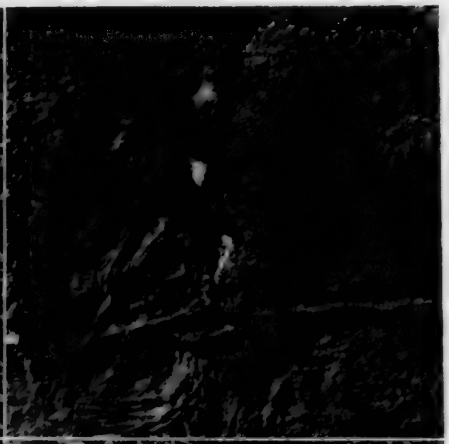

Fig. 9.

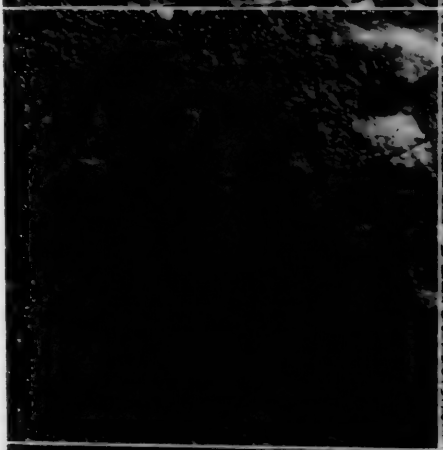

Fig. 10.

Fig. 11.

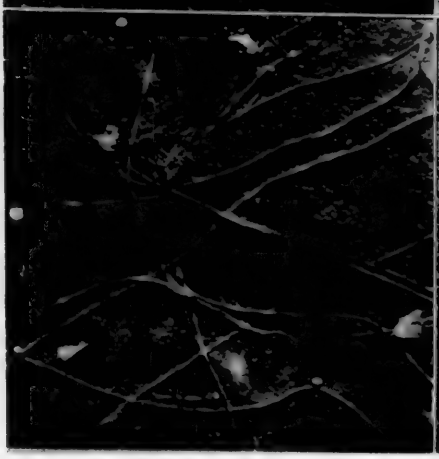

Fig. 12.

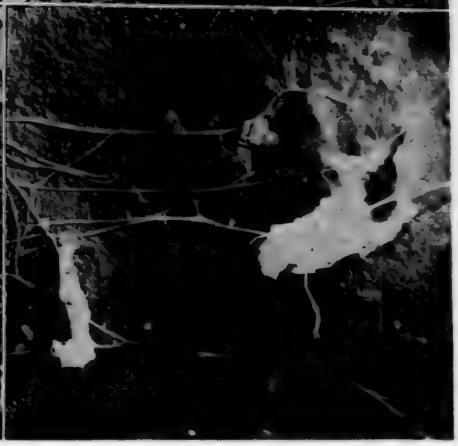

Fig. 7.—Collagen fibers from the dentinal matrix; dentin etched 5 seconds with 0.05N HCl. (×12,100.)

Fig. 8.—Collagen fibers from the dentinal matrix; dentin etched 5 seconds with 0.05N HCl. (×13,300.)

Fig. 9.—Replica of dentinal matrix; dentin etched 5 seconds with 0.05N HCl. (×8,700.)

Fig. 10.—Fine fibers isolated from dentin; dentin etched 10 seconds with 0.4N citric acid. (×10,200.)

Fig. 11.—Fine fibers isolated from dentin; dentin etched 10 seconds with 0.4N citric acid. (×12, 000.)

Fig. 12.—Fine fibers associated with a dentinal fiber; dentin etched 5 seconds with 0.05N HCl. (×8,150.)

Dentinal matrix.—On the basis of the optical examination of decalcified sections the matrix of mature dentin has been described as a calcified network of collagenous fibrils.[4-9] It has been difficult in our studies to demonstrate structural detail in the intertubular areas of replicas of etched dentin sections. In most instances these regions have shown no fine detail other than a uniform granularity (Figs. 1 to 4). Sections lightly etched from 1 to 5 seconds with 0.05N HCl have, however, sometimes yielded replicas containing small numbers of fibers which apparently are collagen. They have cross striations ca. 640A apart and otherwise resemble collagen from tendon and other types of connective tissue (Figs. 7 and 8). In addition, many areas have been noted which appear to be replicas of masses of these fibrils retained in the section but which are well enough replicated to reproduce their characteristic cross striations (Figs. 8 and 9). Most of these fibrils have been noted in cross rather than longitudinal sections through dentin. They are very thin filaments (ca. 0.05 micron) and are thus individually far below the resolving power of the optical microscope. The structures that have been observed by direct optical microscopy of decalcified cross sections[4-9] have presumably been either clusters of several of these fibrils or thicker fibrils that may be present.

Other fine fibers.—In addition to the dentinal fibers with their enclosures and branches, and the collagenous fibrils of the matrix, large numbers of extremely fine fibers, different in appearance from any of the others, have been observed in almost all types of preparation. These fibers vary in diameter, from 0.01 to 0.1 micron, and are seen singly, in parallel bundles, or twisted together like ropes (Figs. 10, 11, 12). They often appear in close association with the dentinal fibers (Fig. 12), and as often are found lying separately on regions of the replicas between tubules. It has thus far not been possible to determine if these fibers are a part of the matrix, or if they constitute part of the contents of the tubule system which have been torn out.

SUMMARY

Three types of fibers have been observed during the electron microscopic study of dentin. They are the large, thin-walled dentinal (Tomes') fibers, fibrils of the matrix, which show the characteristic 640A repetitions of collagen, and very fine fibers of unknown character.

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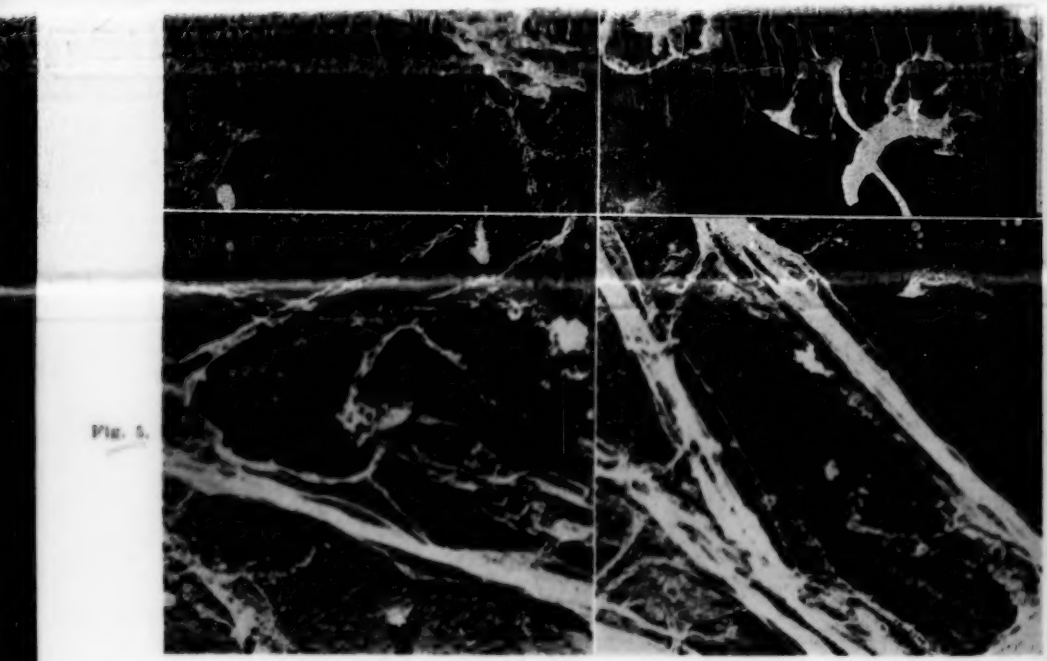

Fig. 5.

Fig. 6.

Fig. 1.—Lightly-etched dentin; etched 5 seconds with 0.1N citric acid. (×5,500.)
Fig. 2.—Dentinal fibers; dentin etched 10 seconds with 0.1N HCl. (×5,350.)
Fig. 3.—Outer wall of a dentinal fiber; dentin etched 3 seconds with 0.1N HCl. (×5,550.)
Fig. 4.—Branched dentinal fibers; dentin etched 10 seconds with 0.1N HCl. (×5,600.)
Fig. 5.—Dentinal fiber torn open; dentin etched 5 seconds with 0.8N citric acid. (×7,350.)
Fig. 6.—Dentinal fiber torn open; dentin etched 5 seconds with 0.8N citric acid. (×7,350.)

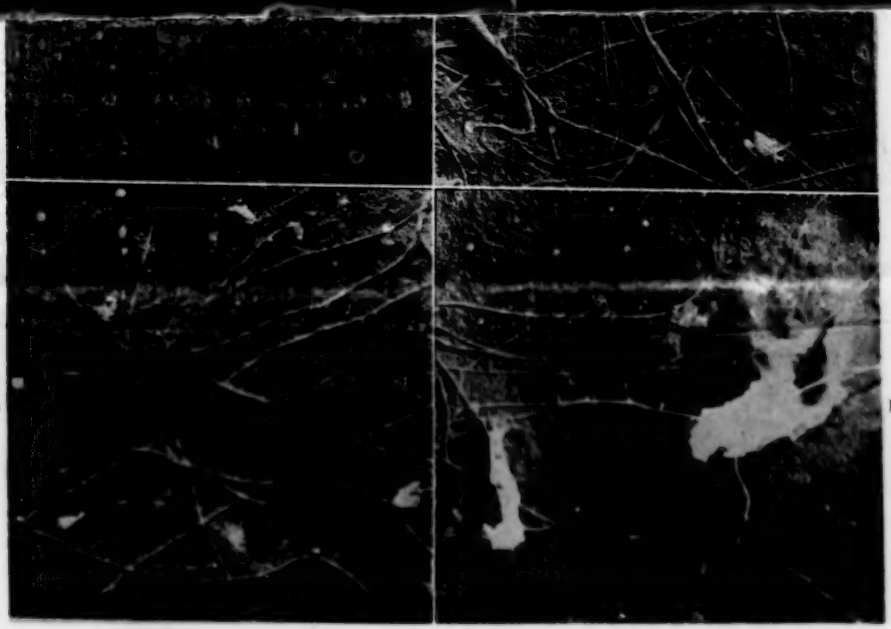

Fig. 11.

Fig. 12.

Fig. 7.—Collagen fibers from the dentinal matrix; dentin etched 5 seconds with 0.05N HCl. (×12,100.)

Fig. 8.—Collagen fibers from the dentinal matrix; dentin etched 5 seconds with 0.05N HCl. (×13,300.)

Fig. 9.—Replica of dentinal matrix; dentin etched 5 seconds with 0.05N HCl. (×8,700.)

Fig. 10.—Fine fibers isolated from dentin; dentin etched 10 seconds with 0.4N citric acid. (×10,200.)

Fig. 11.—Fine fibers isolated from dentin; dentin etched 10 seconds with 0.4N citric acid. (×12, 000.)

Fig. 12.—Fine fibers associated with a dentinal fiber; dentin etched 5 seconds with 0.05N HCl. (×8,150.)

Dentinal matrix.—On the basis of the optical examination of decalcified sections the matrix of mature dentin has been described as a calcified network of collagenous fibrils.[4-9] It has been difficult in our studies to demonstrate structural detail in the intertubular areas of replicas of etched dentin sections. In most instances these regions have shown no fine detail other than a uniform granularity (Figs. 1 to 4). Sections lightly etched from 1 to 5 seconds with 0.05N HCl have, however, sometimes yielded replicas containing small numbers of fibers which apparently are collagen. They have cross striations ca. 640A apart and otherwise resemble collagen from tendon and other types of connective tissue (Figs. 7 and 8). In addition, many areas have been noted which appear to be replicas of masses of these fibrils retained in the section but which are well enough replicated to reproduce their characteristic cross striations (Figs. 8 and 9). Most of these fibrils have been noted in cross rather than longitudinal sections through dentin. They are very thin filaments (ca. 0.05 micron) and are thus individually far below the resolving power of the optical microscope. The structures that have been observed by direct optical microscopy of decalcified cross sections[4-9] have presumably been either clusters of several of these fibrils or thicker fibrils that may be present.

Other fine fibers.—In addition to the dentinal fibers with their enclosures and branches, and the collagenous fibrils of the matrix, large numbers of extremely fine fibers, different in appearance from any of the others, have been observed in almost all types of preparation. These fibers vary in diameter, from 0.01 to 0.1 micron, and are seen singly, in parallel bundles, or twisted together like ropes (Figs. 10, 11, 12). They often appear in close association with the dentinal fibers (Fig. 12), and as often are found lying separately on regions of the replicas between tubules. It has thus far not been possible to determine if these fibers are a part of the matrix, or if they constitute part of the contents of the tubule system which have been torn out.

SUMMARY

Three types of fibers have been observed during the electron microscopic study of dentin. They are the large, thin-walled dentinal (Tomes') fibers, fibrils of the matrix, which show the characteristic 640A repetitions of collagen, and very fine fibers of unknown character.

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